

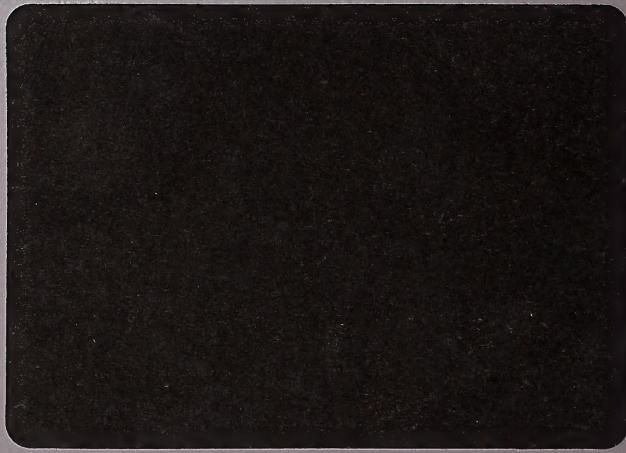
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**RESPONSE OF BARLEY, FIELD  
PEA, CANOLA AND TREE  
SEEDLINGS TO ETHYLENE  
EXPOSURE**

**Alberta's Ethylene/Crop  
Research Project**

**ALBERTA  
RESEARCH  
COUNCIL**





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EXPOSURE**

**Alberta's Ethylene/Crop  
Research Project**

**REPORT III**

**Daniel J. Archambault**

**and**

**Xiaomei Li**

**Alberta Research Council**

**Vegreville, Alberta**

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## PREFACE

### *The Alberta Ethylene Crop Research Project*

This is the last of a series of reports issued by the *Alberta Ethylene Crop Research Project*.

The *Alberta Ethylene Crop Research Project* was initiated by industry and government in response to concerns regarding the potential effects of ethylene emissions from industrial facilities on field crops. The research is jointly sponsored by the petrochemical industry and the Alberta Government, and the research is being conducted at the Alberta Research Council in Vegreville, Alberta. This three-year project began in 1997.

The *Alberta Ethylene Crop Research Project* is designed to meet two objectives:

- To provide scientific information to Alberta Environment for further development of ambient air quality guidelines for ethylene.
- To provide the petrochemical industry and communities adjacent to petrochemical facilities with scientific information for site-specific risk assessments.

The project has been designed to investigate crop responses to ethylene in a staged manner. The first stage is a screening stage in which the relative sensitivity of a number of crops and cultivars (at a number of growth stages) to ethylene is assessed. The second stage is an investigation of the most sensitive crops at their sensitive growth stages to high, short-term exposures to ethylene ("acute" responses). The third stage is a study of the responses of these same sensitive crops to long-term, relatively low concentrations of ethylene ("chronic" responses), as continuous or intermittent exposures.

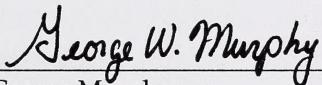
This report presents the results of short-term, long-term and intermittent exposure studies.



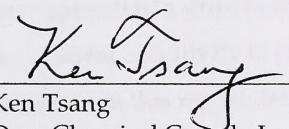
## CLOSURE

The completion of this phase of the research project concludes the research initiative. This study provides scientific information and data that can be used by Alberta Environment to further develop ambient air quality guidelines for ethylene, and by the petrochemical industry and communities adjacent to petrochemical facilities to conduct site-specific risk assessments.

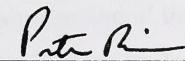
The Management Team for this project was composed of representatives of the industries and government agencies that provided the funding for the project. Additionally, the Management Team was responsible for ensuring that the project met the established objectives. By signing below, members of the Management Team indicate that the objectives have been met.



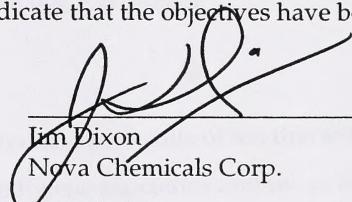
George Murphy  
Alberta Environment



Ken Tsang  
Dow Chemical Canada Inc.

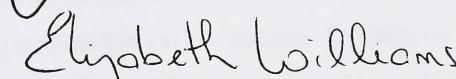


Peter Bieman  
AT Plastics

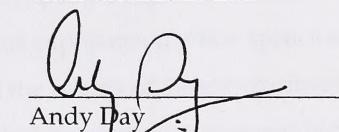
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Jim Dixon  
Nova Chemicals Corp.

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Elizabeth Williams  
Shell Chemicals Ltd.

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Andy Day  
Celanese Canada

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## SUMMARY

### Project Goals

The Alberta Ethylene Crop Research Project was a multi-stakeholder initiative to investigate the response of selected cultivars of agricultural crops of interest to Alberta producers to ethylene exposure. The goals of the project were to:

- Provide scientific information to Alberta Environment for further development of ambient air quality guidelines for ethylene, and to
- Provide the petrochemical industry and communities adjacent to petrochemical facilities with scientific information for site-specific risk assessment.

### Approach

This research project was conducted in three phases. The results of the first two phases, development of a growth chamber system for ethylene exposures and the evaluation of crop and cultivar sensitivity to ethylene, have been presented in earlier reports. The results presented in this report describe the responses of barley cv. Harrington, canola cv. Quantum and field pea cv. Carrera to a range of ethylene exposure regimes and concentrations. An examination of the effects of short-term ethylene exposure on white spruce and lodgepole pine seedlings was also conducted. On the basis of the results of these experiments, an examination of mathematical evaluations of responses to ethylene exposures was conducted to assist in the interpretation and application of the results in guideline development and risk assessment. For the purpose of this study, effects of ethylene on yield were deemed more important than effects on vegetative characteristics. This is the third and final phase of the project.

### Summary of Conclusions

1. No significant effects of short-term exposure to ethylene, up to 1200 ppb for 12 hours, were observed on vegetative and reproductive characteristics in barley, field pea and canola. Similarly, no effects of short-term exposures to ethylene, up to 1200 ppb for 12 hours, were observed on seed germination, seedling vigour, growth in the rapid growth phase, and seedling marketability in lodgepole pine and white spruce.

- 2. Several vegetative and reproductive symptoms of ethylene exposure were observed in the three crop species studied in long-term exposure.** Ethylene symptoms appeared earliest in field pea and symptoms were most noticeable. Photographs of these symptoms are contained within this report.
- 3. Barley cv. Harrington is more sensitive to ethylene exposure than field pea cv. Carrera and canola cv. Quantum.** For this reason, the majority of the experiments were conducted using barley.
- 4. The response of plants to long-term ethylene exposure depends on both concentration and length of exposure.** Minimum ethylene concentrations and length of exposure that are required to cause an effect are species-specific. Barley yields were reduced significantly when plants were exposed to 50 ppb ethylene for 3 days, while no significant decrease in seed yield of field pea was observed when plants were exposed to 50 ppb ethylene for up to 26 days.
- 5. The ability to recover from the effects of long-term ethylene exposure is species-specific.** Significant reductions in yield of field pea were observed in plants exposed to ethylene at concentrations above 100 ppb for 16 days, however, given sufficient time following treatment, complete recovery of yield in field pea was observed. Barley and canola did not appear to have the recovery abilities of field pea.
- 6. The sensitivity of barley varies with the time of day of ethylene exposure.** Barley was most sensitive to ethylene during the middle of the day [between 10:00 a.m. and 4:00 p.m.].
- 7. Exposure of barley plants to an ethylene pattern derived from ambient air monitoring data from the worst month of exposure seen at a petrochemical facility in Alberta over a three year period had no significant effect on yield.** Exposure was at the most sensitive stages of barley growth and reproductive development.

8. Intermittent exposure experiments indicated that exposure of barley to five intermittent exposures of 250 ppb ethylene caused a reduction in yield. Significant experimental difficulties were encountered in this part of the work. Intervals as long as 5 days between exposures were not sufficient to allow for complete recovery.
  
9. The application of mathematical expressions of dose to the data indicate that the interim 6-hour Alberta Air Quality Ethylene Guideline ( $120 \mu\text{g m}^{-3}$  or 104 ppb) appears to be conservatively protective of barley, canola and field pea from yield reductions due to short-term ethylene exposure. The interim 30-day Alberta Air Quality Ethylene Guideline ( $50 \mu\text{g m}^{-3}$  or 44 ppb) appears to be protective of yield effects in canola and field pea; however, yield effects may occur in barley at levels below the 30-day interim Alberta Air Quality Ethylene guideline ( $50 \mu\text{g m}^{-3}$  or 44 ppb).

## **ACKNOWLEDGEMENTS**

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Finally, the authors wish to acknowledge the contributions of research team members: Dr. Paul Sharma, Dr. John O'Donovan, Dr. Abdur Rashid, Dr. Aziz Khan, Richard Milner, Tadeusz Kazmierczak, Henry Bertram and Brian Serink and technicians: Sharon Pappin-Willianen, Karen Sorensen, Dayna McIntyre, Marlene Boissoneau and Jim Storey for their support and dedication.

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## **1. INTRODUCTION**

Ethylene gas ( $C_2H_4$ ) has profound effects on a diverse array of plant growth and development processes, including germination, flowering, senescence, abscission, fruit ripening and yield (Abeles et al. 1992). Plants produce ethylene in response to stresses, such as water stress, wounding, or other environmental stresses (Melhorn and Wellburn 1987; Taylor and Gunderson 1986; O'Donnell et al. 1996). As a constituent of air, ethylene can also be a phytotoxic pollutant (Squier et al. 1985) with serious consequences primarily in confined areas such as those used for fruit storage. As an indirect or secondary phytotoxicant produced in response to stress agents such as  $SO_2$  and  $O_3$ , ethylene plays a significant role in the response of plants to air pollutants (Abeles and Heggestad 1973; Abeles 1982). In the field, ethylene from anthropogenic sources is thought to be less effective than stress-induced endogenous ethylene because the concentrations are low and exposure episodes tend to be transient. However, emissions from point sources such as those from industrial facilities might have negative effects on plants.

The recent expansion of the ethylene industry in Alberta has led to increased concerns over the potential effects of fugitive emissions from localized sources on the performance of adjacent crops. In order to address these concerns, this research project was initiated and focuses on the potential effects of ethylene on Alberta crops.

In the process of developing an interim guideline for ethylene emissions in Alberta, Alberta Environment (1997) pointed out that a large body of literature has been generated in an attempt to understand the biosynthesis of ethylene in plants and its relation to environmental factors. Because interest in ethylene is primarily as a plant growth regulator, studies have focused on the effects of ethylene at specific plant growth stages, such as flowering, and fruiting and on photosynthesis and stomatal conductance. The concentrations used in these studies ranged from the low ppb range to levels above 10 ppm. Only studies documenting effects of ethylene below 1 ppm were included in Alberta Environment (1997), as ambient levels rarely approach the 1 ppm level. Furthermore, rarely have the effects of ethylene on crop yields been addressed (Reid and Watson 1985).

The sensitivity of plant responses to ethylene has been shown to be species and growth-stage specific. The determination of the effects of ethylene emissions on crops should therefore focus on sensitive species and growth stages. Using ethephon as a screening tool, Archambault and Li (1999) studied eight cultivars of five crop species, considered important in Alberta now and in the future, and seven growth stages for each of the species/cultivars. Based on the results of these studies, the three most sensitive crop species/cultivars and the most sensitive growth stage for each species were selected to conduct detailed studies of the effects of long-term exposure to ethylene using a growth chamber exposure system (Li and Archambault 1999).

The present report describes the results of ethylene exposure experiments conducted using barley, field pea, canola and tree seeds/seedlings. Following this introduction, the second section describes plant growth conditions, general exposure regimes and assessment procedures for various plant parameters. Sections 3 to 8 present the results of the following six groups of exposure experiments, which were designed to address specific questions:

- Response to short-term exposures: to determine the concentration threshold at which short-term exposures ( $\leq$  12 hours) of ethylene would cause significant effects on the parameters measured in crops and tree seeds/seedlings;
- Response to long-term exposures: to determine the concentration threshold at which long-term exposures (73 days) to ethylene would cause significant effects on the parameters measured in crops;
- Varying long-term exposure duration: to determine the critical duration of exposure at 50 ppb that would cause significant effects on yield in plants exposed to ethylene during a sensitive stage;
- Time of day exposure: to test whether the sensitivity of barley to ethylene varied with time of day.
- Demonstration experiment: to test whether a demonstration pattern based upon monitoring data for a 30-day period at a site in Joffre, Alberta would induce responses in barley;
- Exposure interval length and plant recovery: to test whether barley plants will recover from ethylene injury if the interval length between exposures is long enough.

In the last section, methods of integrating the data generated in this project were investigated in order to assist in the understanding of crop responses to ethylene exposure. This section focuses on the analysis of the results from continuous exposure experiments, i.e. short- and long-term exposure experiments. This discussion also served to compare the results from short- and long-term exposure experiments from the present study with the data used for the development of the interim Alberta Ethylene Guideline (Alberta Environment 1997) and to assess the suitability of using the Log Sum Dose function developed by Randall D. Jones (pers. comm.), Ontario Ministry of Environment and Energy, and the log-log dose-response function developed through this study to describe the responses of Alberta crops to ethylene exposures.

## **2. PLANT GROWTH CONDITIONS AND ASSESSMENTS**

### **2.1 Plant Selections**

Three crop species were selected for the present study by the Technical Advisory Committee after recommendation from ARC's research team based on screening experiments performed using ethephon to determine the most sensitive species and growth stages. Along with plant sensitivity to ethephon, other criteria such as plant growth and care requirements and relative success at producing the plant organs of agricultural value were also considered (Archambault and Li 1999). One representative of each of cereals, oilseeds and legumes was selected. The effects of short-term exposures of ethylene on germination and early seedling growth in two conifer species grown in a nursery adjacent to an ethylene plant in Joffre, Alberta were also studied.

The crop species and cultivars chosen for the study of the effects of exposure to ethylene were:

- i) Cereal:  
barley (*Hordeum vulgare*) cv. Harrington (2 row).
- ii) Legume:  
field pea (*Pisum sativum*) cv. Carrera.
- iii) Oilseed:  
canola (*Brassica napus*) cv. Quantum.

The tree species studied were:

- i) White spruce (*Picea glauca*).
- ii) Lodgepole pine (*Pinus contorta*).

### **2.2 Growth Medium, Containers and Preparation of Plant Materials**

All crops were grown in greenhouses in 20 cm (diameter) plastic pots containing 3 - 4 kg of a standard greenhouse soil mixture consisting of equal volumes of sand, soil, peat and vermiculite at plant densities of 5, 4 and 3 plants per pot for barley, field pea and canola, respectively. The growth medium was initially fertilized with 1.6 g dolomitic limestone (52% CaCO<sub>3</sub> and 41% MgCO<sub>3</sub>) and 1.0 g superphosphate (45% available P<sub>2</sub>O<sub>5</sub>) per pot (ARC standard procedure). Following emergence, the growth medium was fertilized on a weekly basis using NH<sub>4</sub>NO<sub>3</sub> and/or KH<sub>2</sub>PO<sub>4</sub> to maintain total available N at approximately 300 ppm and P at

approximately 100 ppm. Nitrogen and phosphorus requirements were determined based on weekly soil testing performed with the same crops during the ethephon screening experiments. In the case of canola, sulfur was added in a 6:1 ratio with nitrogen (6N:1S). Micronutrients were also added periodically using a  $\frac{1}{4}$  strength modified Hoagland's solution containing only micronutrients. Plants were watered twice-daily using tap water so as to maintain the soil moist, avoiding plant wilt or waterlogging.

All tree seeds were sown into soil plugs produced by K&C Silviculture, Joffre, Alberta. Information pertaining to the preparation of soil plugs is proprietary and will not be given in this report. The plugs contained macro and micronutrients. For germination experiments, at two-day intervals and for each species, 6 styrofoam trays each containing 56 plugs were sown at a rate of 4 and 6 seeds per plug for pine and spruce, respectively. The seeded plugs were transported in a cargo van from K&C Silviculture to ARC, labeled and placed into growth chambers for subsequent exposure to ethylene.

For experiments on effects of ethylene on seedlings in the rapid growth phase, trays of seeded plugs were prepared as described above and seedlings were grown at K&C Silviculture until they reached the appropriate phase (approximately 3 months old). They were then moved to ARC in a cargo van and placed into a greenhouse that was set-up to approximate the greenhouse conditions at K&C Silviculture. Groups of seedlings from each species were moved to the exposure chambers, exposed to ethylene and moved back to the greenhouse at two-day intervals.

Following a one-week equilibration period, half of the seedlings were returned to K&C Silviculture for subsequent growth over approximately 3 months. The remaining seedlings were grown at ARC for the same length of time. This allowed us to assess whether transportation caused effects that would mimic ethylene injury and to verify whether the differences in plant growth conditions between ARC and K&C Silviculture subsequent to exposure affect the results.

## **2.3 Pest Control**

Several biological controls and pesticides were employed to control insect pests and diseases as required following manufacturers' recommended methods. Aphid lions, aphidoletes, cucumeris, green lacewings, hypoaspis, ladybugs, and Nemasys (Westgro) were used to control insect pests. Chemical pesticides were used where biological controls were not sufficiently effective. Benlate (Dupont Canada Inc.), Kumulus DF (BASF Canada Inc.), Tilt (Novartis Crop Protection Canada Inc.) and Vitavax (Uniroyal Chemical Ltd.) were used to control disease organisms. Cygon 240 EC (United Agri Products), Impower (Plant Products Co. Ltd.), Pirimor (Chipman Inc.), Safer's Insecticide Soap (Safer Ltd.) and Nicotine Fume (Plant Products Co. Ltd.) were used to control insect pests. Upon detection of pest outbreaks, a method of pest control was selected and applied evenly to all plants.

Over the length of the study and by the end of short-term exposure experiments, the efficacy of biological controls and pesticides for the control of aphids decreased to unacceptable levels. Fumigation using nicotine became essential to control aphids at the start of long-term exposure experiments on barley. Nicotine treatments were applied at nighttime in the greenhouse before and/or after exposures to ethylene as required. Ethylene concentration in the greenhouse during fumigation was measured by sampling air in the center of the greenhouse at approximately one meter above the nicotine bomb. Ethylene concentrations peaked at 365 ppb approximately 16 minutes after ignition of the nicotine bomb and decreased exponentially to less than 30 ppb two hours after ignition. Ethylene concentrations continued to decrease to non-detectable levels after 13.5 hours. This resulted in an approximate equivalent dose of 120 ppb for 1.5 hours, an ethylene dose that was shown not to cause measurable effects on vegetative or reproductive parameters in barley (section 3).

## **2.4 Greenhouse Conditions**

Light, temperature and relative humidity were monitored using quantum sensors (Li-190SA, LI-COR Corp.) attached to a light meter (Li-250, LI-COR Corp.) and temperature/humidity probes (HMP35A, Campbell Scientific). A photoperiod of 16 hours light and 8 hours dark was maintained for all experiments using natural light supplemented by sodium halide lights. Light intensities at mid-canopy were determined to vary between 185 and 810  $\mu\text{mol m}^{-2} \text{s}^{-1}$  depending

on natural light levels, time of day and time of year. Temperatures ranged from 19 to 33°C in the daytime and 15 to 22°C at night. Relative humidity ranged from 20 to 90%. In the case of tree seed germination and early growth, relative humidity was maintained between 70 and 100% using a misting system controlled by a timer.

## 2.5 Exposure Chamber Conditions

Light was measured at mid-canopy using a quantum sensor (Li-190SA, LI-COR Corp.) attached to a light meter (Li-250, LI-COR Corp.) and light levels in all growth chambers were adjusted to uniformity by adjusting the height of the banks of lights. Temperature was monitored using thermocouples mounted within the chambers and recorded on built-in chart recorders original to the growth chambers. Relative humidity was monitored using humidity probes (Vaisala) mounted in ports in two of the six growth chambers and logged to a Pro-logic controller (PLC). A photoperiod of 16 hours light and 8 hours dark was maintained for all experiments using fluorescent lights. Light intensities at mid-canopy were maintained between 285 and 325  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Temperatures were approximately 23°C in the daytime and 15°C at night. Relative humidity ranged from 40 to 60%.

## 2.6 Exposures

The exposure system used in the present study has safeguards that allow for the verification of proper function. The mass flow controllers have electronic read-backs that were compared to calculated values. These were checked by the operator on a regular basis during exposure. The ethylene flows and airflows were continuously measured and logged so that the data could be recalled and verified in the event that the system malfunctioned. Theoretical ethylene concentrations can be calculated from these data. While this does not give direct measurements of ethylene concentrations in the chambers, by combining this with the gas chromatography (GC) measurements we were able to detect malfunctions that would result in ethylene concentrations beyond or below acceptable limits (10% of target). If no malfunctions were detected using these indicators, the assumption was made that the actual ethylene concentration was on target. The exposure concentration was then reported as the target concentration. This was done because the accuracy of the exposure system was shown to be greater than that of the GCs (Li and Archambault 1999). In the case where any or all of the

safeguards ‘alarmed’ the operator of the possibility of system malfunction, the GC measurements were taken as being correct.

For all experiments, plants were grown in the greenhouse until the appropriate stage was reached at which time they were transferred to the exposure system and left for one day to acclimate prior to the start of exposures. Plants were moved back to the greenhouse and grown to maturity after exposures were complete.

## 2.7 Measurements/Assessments

### 2.7.1 Qualitative Assessments

Plants were observed for visual symptoms during and following exposure to ethylene. Photographic records were kept.

### 2.7.2 Quantitative Assessments

#### 2.7.2.1 Barley

Vegetative characteristics:

- Height of the three tallest plants per pot was measured.
- Number of tillers per pot was counted.
- Total vegetative above-ground biomass was determined.
- Total root biomass per pot was determined. This was done by soaking the pots in a bucket of water following excision of the above-ground biomass and manually removing the soil by spraying the root mass with water followed by the removal of the remaining soil debris using forceps. The roots were dried in a drying oven at 70°C and weighed.

Reproductive characteristics:

- Total numbers and weights of all heads per pot were determined.
- Total numbers and weights of all seeds per pot were determined.
- Weight per thousand seeds was calculated.
- Ground seed samples were analyzed for protein, calcium and phosphorus by NIR or wet chemistry methods at the Soil and Crop Diagnosis Centre of Alberta Agriculture in Edmonton, Alberta.

- Whole seeds were analyzed for caloric content per gram of material using Bomb Calorimetry (ASTM Standards for Bomb Calorimetry and Combustion Methods 1972) on site at ARC, Vegreville.

#### 2.7.2.2 *Field Pea*

Vegetative characteristics:

- Height of the tallest plant per pot was measured.
- Thickness of the stem at the third internode from the shoot apex was measured.
- Total above-ground biomass per pot was determined.
- Total root biomass per pot was determined as described above.

Reproductive characteristics:

- Number of yellow and green pods per pot were counted and total pods calculated.
- Total weights of all pods and seeds per pot were measured.
- Weight per thousand seeds was calculated.
- Ground seed samples were analyzed for protein, calcium and phosphorus by NIR or wet chemistry methods at the Soil and Crop Diagnosis Centre of Alberta Agriculture in Edmonton, Alberta (See Archambault and Li 1999).
- Ground seed samples were analyzed for caloric content per gram of material using Bomb Calorimetry (ASTM Standards for Bomb Calorimetry and Combustion Methods 1972) on site at ARC, Vegreville.

#### 2.7.2.3 *Canola*

Vegetative characteristics:

- Height of the tallest plant per pot was measured.
- Total above-ground biomass (vegetative and reproductive) was determined.
- Thickness of the stem at 10 cm from the soil was measured.
- Total root biomass per pot was determined as described above.

Reproductive characteristics:

- Total number and weights of seeds per pot were determined.
- Weight per thousand seeds was calculated.

- Whole seeds were analyzed for total oil, protein, calcium and phosphorus content using NIR or wet chemistry methods at the Soil and Crop Diagnosis Centre of Alberta Agriculture in Edmonton, Alberta.

Seed quality analyses were not possible for samples from treatments causing extreme losses in yield.

#### 2.7.2.4 Tree Seeds/Seedlings

For germination experiments, the number of emerged seeds per plug were counted and recorded at approximately 2-day intervals from the time of exposure. Vigour of each seedling was assessed two weeks following exposure according to standard K&C Silviculture protocol. Seedlings were classified according to Figure 2.1 with vigour class 1 representing the greatest level of vigour. The number of individuals in each vigour class was tallied and the number of individuals in vigour class 1 was compared between treatments.

Seedlings treated at the rapid growth phase were grown for an additional 14 weeks following exposure to ethylene. To reduce variability caused by edge effects, only the 8 plugs at the center of each seedling tray were assessed. At harvest time, height, number of branches and health of the primary meristem were assessed. Seedlings were excised at ground level to separate above-ground and below-ground biomass. The above-ground biomass and the remaining plug containing roots were dried at 70°C and weighed.

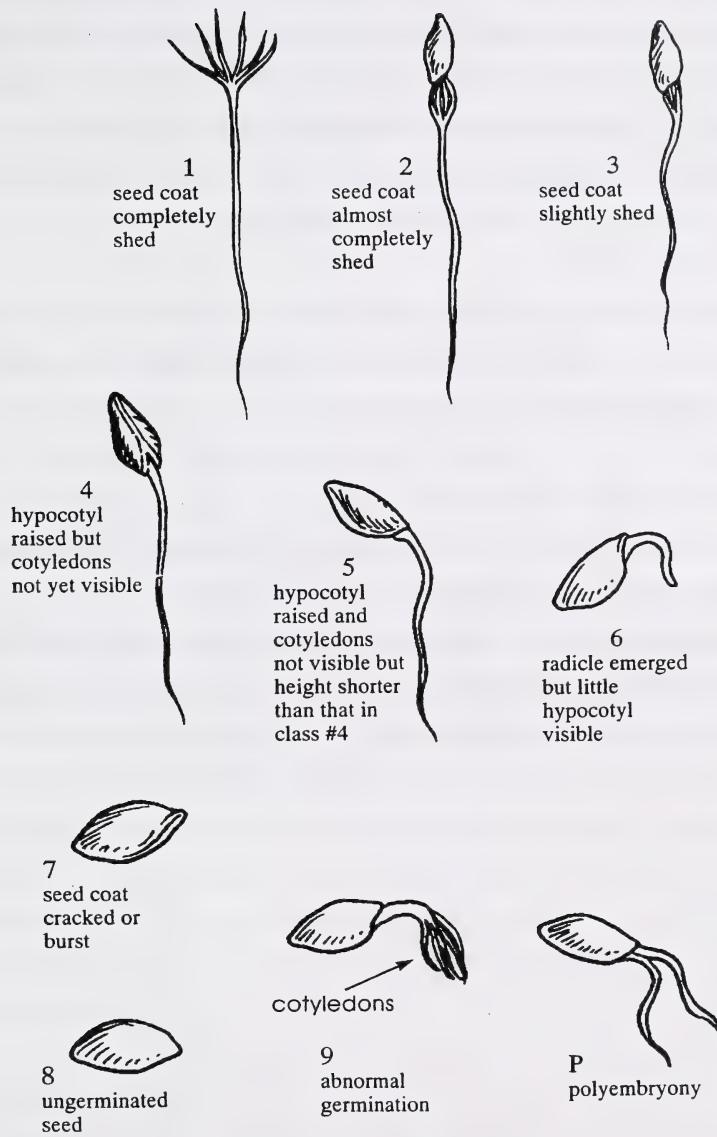


Figure 2.1. Tree seedling diagrams used to establish seedling vigour. Two weeks after exposure to ethylene, each seedling was given a vigour ranking (1 to 9). Rankings were used to compare mean vigour values and treatment effects.

## 2.8 Data Analysis and the 'Dose' Function

Treatment effects were computed using ANOVAs. Means were ranked using Duncan's Multiple Range test where the ANOVA detected treatment effects. Regressions were used to describe the relationships between ethylene exposure, including concentration and duration, and various plant parameters. Where regressions were used, slopes were analyzed for deviance from zero. If the slope did not differ significantly from zero, it was deemed that no significant relationship existed between ethylene exposure and the measured parameter.

Attempts were made to describe the response of crops to both short- and long-term exposures to ethylene through the use of dose functions defined as the combined contributions of both duration of exposure ( $t$ ) and concentration ( $C$ ) of ethylene.

$$[2-1] \quad \text{Dose} = f(C,t)$$

Throughout this report, various 'dose' function forms are employed to summarize data and to help in the interpretation of data. In section 3 of this report, dose is defined as the product of concentration of ethylene in ppb and the duration of exposure in hours.

In section 9, a more detailed dose was defined in the log-log dose-response function, which was then compared to the Log Sum Dose function developed by Randall D. Jones (pers. comm.) to describe the response of petunia to ethylene exposure.

### **3. RESPONSES TO SHORT-TERM EXPOSURE**

#### **3.1 Introduction**

The purpose of these experiments was to determine the concentration threshold at which short-term exposures of ethylene would cause significant effects on vegetative and reproductive parameters. For the crop species studied, while several measurements of vegetative characters were performed, the emphasis was placed on potential effects of ethylene exposure on seed yield. This was done to reflect the importance of seed yield to producers. Throughout this study, sensitivity of seed yield to ethylene was used to establish relative sensitivity of the crop species. In the case of the tree seed/seedling experiments, however, emphasis was placed on potential effects of ethylene exposure on seedling marketability. Vegetative characters deemed important in marketability were therefore measured. Tree seed/seedling studies arose from concerns from tree nursery operators in Joffre, Alberta.

For the purpose of this study, short-term exposures were defined as being equal to or less than 12 hours in duration. Exposures were performed at the most sensitive stage of plant growth as determined in previous studies (Archambault and Li 1999). Experiments were conducted in four parts, with each part including one exposure time and six concentrations (a total of 4 exposure times and 6 concentrations). To minimize the effects of conducting the four parts of the experiment at different times, each part of the experiment was conducted at approximately 2-day intervals with only 6 days between parts 1 and 4 (Figure 3.1). At all other times, the plants were under the same environmental conditions. For each exposure duration, a concentration of ethylene was assigned to each of the chambers and 6' replicate pots were placed into each chamber. Due to the limited number of chambers, all replicates were within the same chamber.

#### **3.2 Exposure Regimes**

All exposures were performed and verified as described in section 2.5. No system malfunctions occurred in the trials reported here, therefore the target concentrations are used. They were: 10, 75, 150, 300, 600 and 1200 ppb. All exposures were conducted in the daytime for 1.5, 3, 6 or 12 hours (Figure 3.1).

### 3.3 Results

A number of preliminary trials were conducted to develop and test the short-term exposure protocols prior to conducting the formal trials described in detail here. The results of the preliminary trials can be found in Appendix I.

In these experiments, exposures to ethylene were staggered over a period of six days with four sets of plants exposed for a specific duration of time (Figure 3.1). For the rest of time, all plants remained in the same greenhouse conditions. This was done because of the limited number of exposure chambers available for the experiments. This design led to possible confusion between effects of the time of treatment and duration of exposure. However, control treatments were used in each section of the experiment so as to be able to separate the possible effects of time of exposure and duration of ethylene exposure. Treatments included six concentrations of ethylene and four durations of exposure and the order of the exposures were randomly selected.

The ANOVA analysis revealed that no significant effects of ethylene concentration and exposure durations were detectable for any of the parameters measured. Therefore, the analyses of interactions between concentration and duration were not performed.

#### 3.3.1. Crops

Linear regressions were used to examine the relationships between several plant parameters and ethylene dose expressed as the product of ethylene concentration in ppb and exposure duration in hours (concentration x duration). These figures also served as a summary of measured parameters over the range of ethylene doses (Figs. 3.2, 3.3, 3.4). In each case, the R values indicated poor relationships between the parameters measured and ethylene dose.

In barley, regressions of seed head number, seed head weights, seed number and seed weights against ethylene dose yielded R<sup>2</sup> values of 0.014, 0.029, 0.010 and 0.009, with regression slopes of -1.179 ( $\pm 2.072$ ), -1.298 ( $\pm 1.599$ ), -12.754 ( $\pm 27.623$ ) and -0.555 ( $\pm 1.271$ ), and P values for 95% confidence intervals of 0.575, 0.425, 0.649 and 0.667, respectively (Fig. 3.2). In field pea, regressions of seed number, seed weights and pod weights against ethylene dose yielded R<sup>2</sup>

values of 0.066, 0.065 and 0.087, with regression slopes of -1.369 ( $\pm 1.099$ ), -1.147 ( $\pm 0.925$ ) and -4.647 ( $\pm 3.205$ ) and P values for 95% confidence intervals of 0.226, 0.228 and 0.161, respectively (Fig. 3.3). In canola, regressions of seed number and seed weights against ethylene dose yielded R<sup>2</sup> values of 0.049 and 0.028, with regression slopes of 1.283 ( $\pm 1.201$ ) and 238.0 ( $\pm 300.2$ ) and P values for 95% confidence intervals of 0.297 and 0.436, respectively (Fig. 3.4). Data for all other parameters are summarized in Tables 3.1 to 3.8.

### 3.3.2. Tree Seeds/Seedlings

Germination began on days 4-5 for pine and day 7-8 for spruce. Germinated seeds were counted approximately every two days. Germination was complete by about day 12 for pine and day 15 for spruce. The number of germinated seeds per plug was determined at the end of the experiment and data was plotted against ethylene dose (concentration x duration).

As in the case of crop species, linear regressions were used to examine the relationships between several plant parameters and ethylene dose expressed as the product of ethylene concentration in ppb and exposure duration in hours. Regressions of seed germination against ethylene dose yielded R<sup>2</sup> values of 0.007 and 0.002 with slopes of 0.076 ( $\pm 0.025$ ) and 0.048 ( $\pm 0.029$ ) and P values for 95% confidence intervals of 0.002 and 0.103 for pine and spruce, respectively (Fig. 3.5). While the P value for regression of germination of pine was significant, the non-significant R<sup>2</sup> value indicated a poor correlation between germination and ethylene dose and the low slope indicated that the effect was small.

At the conclusion of the experiment each seedling was assessed for vigour according to standard K&C Silviculture protocol. The number of seedlings in vigour class 1 (greatest vigour) was plotted against ethylene dose (Fig. 3.6). Regressions of seedling vigour against ethylene dose yielded R<sup>2</sup> values of 0.006 and 0.005 with slopes of -0.675 ( $\pm 1.642$ ) and 0.734 ( $\pm 2.263$ ) and P values for 95% confidence intervals of 0.684 and 0.749 for pine and spruce, respectively, meaning that there was no significant dose effect (Fig. 3.6).

The effects of ethylene exposure on pine and spruce seedlings in the rapid growth phase were also studied. Seedlings were assessed and harvested approximately 14 weeks after short-term

exposures to ethylene. No differences were observed between plants grown at ARC and those grown at K&C Silviculture in the effects of ethylene and therefore we only present results from plants grown at ARC. Mean seedling heights and above-ground biomass were plotted against ethylene dose (Figs. 3.7 and 3.8). Linear regression was used to determine whether ethylene dose caused effects on height and above-ground biomass. Regressions of seedling height against ethylene dose yielded  $R^2$  values of 0.011 and 0.018 with slopes of -0.419 ( $\pm 0.271$ ) and -1.099 ( $\pm 0.550$ ) with P values for 95% confidence intervals of 0.124 and 0.047 for pine and spruce, respectively (Fig. 3.7). Regressions of seedling above-ground biomass against ethylene dose yielded  $R^2$  values of 0.018 and 0.008 with slopes of 0.098 ( $\pm 0.049$ ) and -0.060 ( $\pm 0.046$ ) with P values for 95% confidence intervals of 0.046 and 0.197 for pine and spruce, respectively (Fig. 3.8). While the P value for regression of height of spruce and above-ground biomass of pine were significant, the non-significant R value indicated a poor correlation between these parameters and ethylene dose and the low slopes indicated that the effects were small. The same analyses were performed for all other parameters measured. No significant effects of ethylene dose were found. Data are summarized in Tables 3.9 and 3.10.

### 3.4 Discussion

No significant effects of short-term exposure of ethylene on the parameters measured for crops and tree seeds/seedlings were observed. In these experiments, we measured photosynthetic rates throughout the exposure period and in some cases at intervals following exposure but did not find ethylene effects when plants were exposed to ethylene for up to 12 hours at 1200 ppb (data not shown). Effects of short-term exposures ( $\leq 28$  hours) of ethylene on photosynthesis have been reported for potato, sweet potato, peanut, corn, soybean, tobacco, sunflower, Jerusalem artichoke and green ash (Squier et al. 1985; Govindarajan and Pooviah 1982; Kays and Pallas 1980; Pallas and Kays 1982) but effects on growth have only been measurable in exposures greater than 24 hours where effects on roots of corn (Whalen and Feldman 1988), tomato, rice and white mustard (Konings and Jackson 1979) were observed. Effects of short-term exposures of ethylene on photosynthesis have been shown to be reversible and no measurable effects on plant development could be observed. It appears that longer exposures would be required for alterations in physiological and biochemical processes to manifest themselves into changes in plant morphology, phenology and yield.

### **3.5 Conclusions**

No significant effects of short-term exposure to ethylene, up to 1200 ppb for 12 hours, were observed on the various parameters measured for barley, field pea and canola. Similarly, no effects of short-term exposures to ethylene, up to 1200 ppb for 12 hours, were observed on seed germination, seedling vigour and seedling marketability in lodgepole pine and white spruce. These results suggest that short-term exposures have either no effects on plants or that the effects are reversible. Perhaps longer exposures are required to cause irreversible and measurable effects on plant morphology and yield in the species studied.



Figure 3.1. Sample schedule for short-term exposure experiments using ethylene. Experiments were conducted in four parts, with each part including one exposure duration and six concentrations. To minimize the effects of conducting the four parts of the experiment at different times, each part of the experiment was conducted at approximately 2-day intervals with only 6 days between parts 1 and 4. At all other times, the plants were under the same environmental conditions.

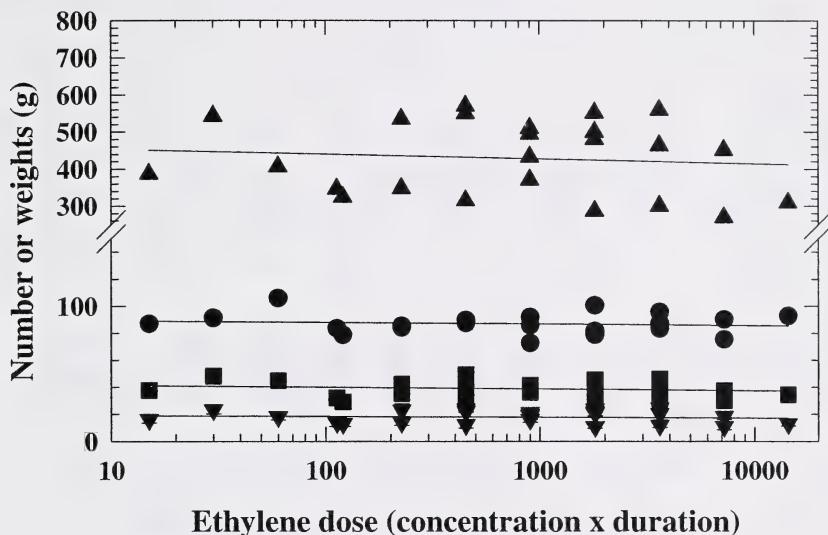


Figure 3.2. Dose (concentration x duration) response in seed numbers (up triangles), seed weights (down triangles), head numbers (circles) and head weights (squares) of barley cv. Harrington. Dose is plotted on a log scale. Values are means where  $N = 5$ . Regressions of seed head number, seed head weights, seed number and seed weights against ethylene dose yielded  $R^2$  values of 0.014, 0.029, 0.010 and 0.009, with regression slopes of  $-1.179 (\pm 2.072)$ ,  $-1.298 (\pm 1.599)$ ,  $-12.754 (\pm 27.623)$  and  $-0.555 (\pm 1.271)$ , and P values for 95% confidence intervals of 0.575, 0.425, 0.649 and 0.667, respectively.

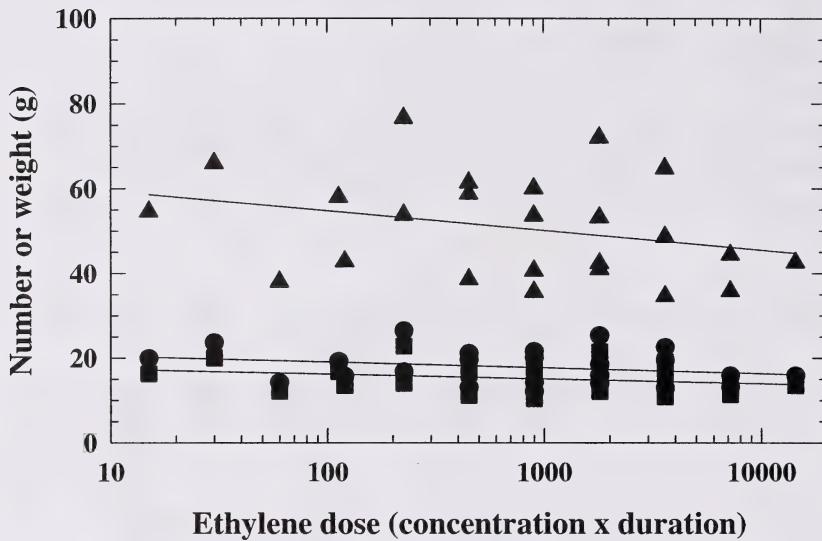


Figure 3.3. Dose (concentration x duration) response in seed number (triangles), seed weight (squares) and pod weight (circles) of field pea cv. Carrera. Dose is plotted on a log scale. Values are means where  $N = 5$ . Regressions of seed number, seed weights and pod weights against ethylene dose yielded  $R^2$  values of 0.066, 0.065 and 0.087, with regression slopes of  $-1.369 (\pm 1.099)$ ,  $-1.147 (\pm 0.925)$  and  $-4.647 (\pm 3.205)$  and P values for 95% confidence intervals of 0.226, 0.228 and 0.161, respectively.

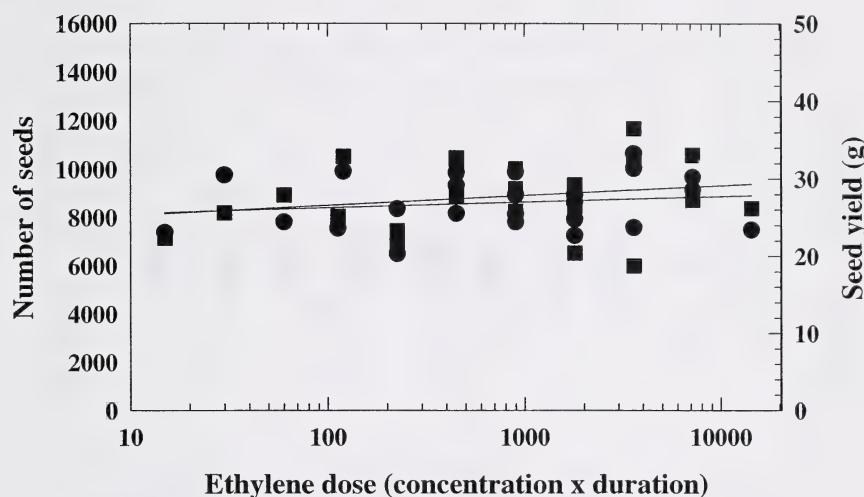


Figure 3.4. Dose (concentration x duration) response in seed number (circles) and seed weight (squares) of canola cv. Quantum. Dose is plotted on a log scale. Values are means where  $N = 5$ . Regressions of seed number and seed weights against ethylene dose yielded  $R^2$  values of 0.049 and 0.028, with regression slopes of 1.283 ( $\pm 1.201$ ) and 238.0 ( $\pm 300.2$ ) and P values for 95% confidence intervals of 0.297 and 0.436, respectively.

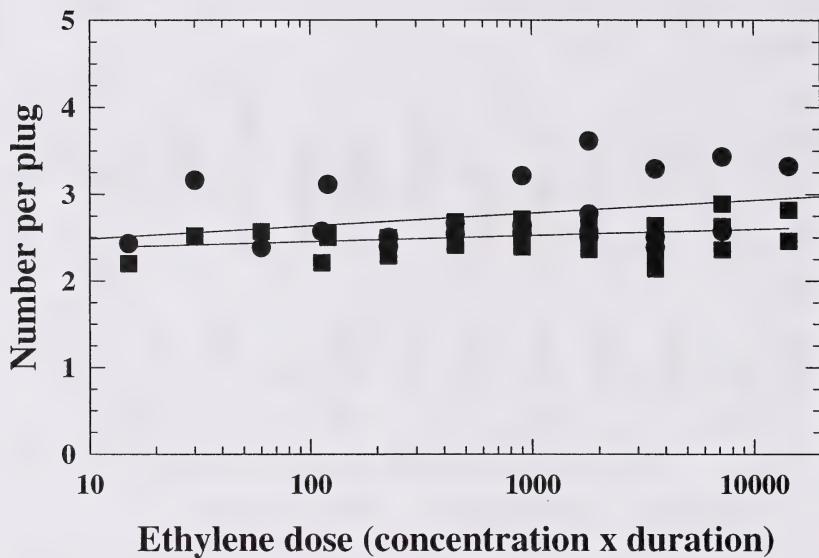
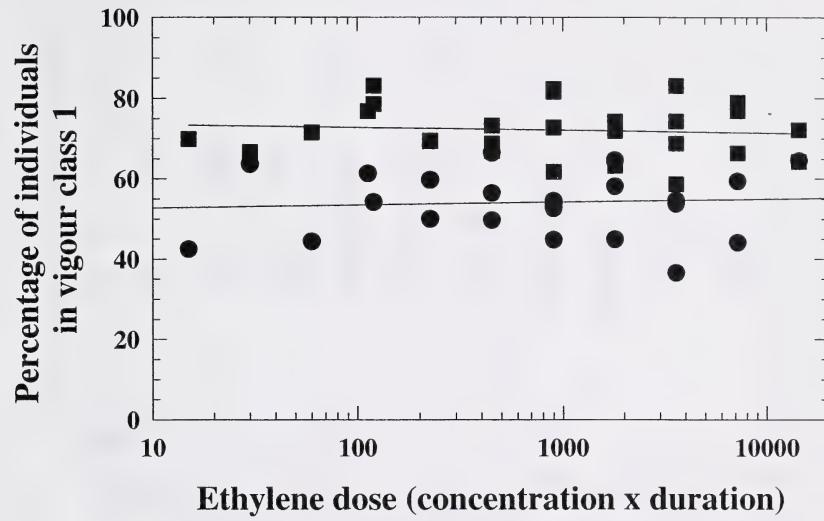


Figure 3.5. Dose (concentration x duration) response in germination of pine (squares) and spruce (circles) exposed to ethylene at the seed stage. Dose is plotted on a log scale. Values are means where  $N = 5$ . Regressions of seed germination against ethylene dose (concentration x time) yielded  $R^2$  values of 0.007 and 0.002 with slopes of 0.076 ( $\pm 0.025$ ) and 0.048 ( $\pm 0.029$ ) and P values for 95% confidence intervals of 0.002 and 0.103 for pine and spruce, respectively.



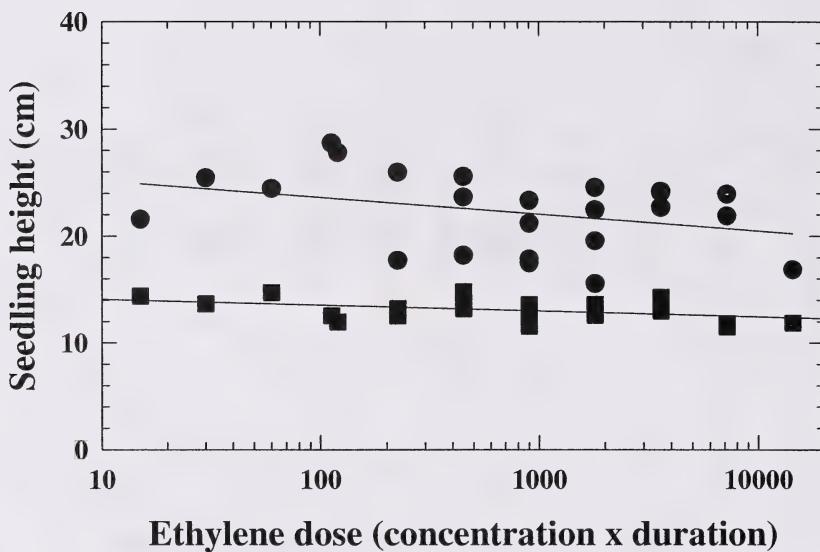


Figure 3.7. Dose (concentration x duration) response in seedling heights of pine (squares) and spruce (circles) exposed to ethylene at the rapid growth phase. Dose is plotted on a log scale. Values are means where  $N = 8$  to 10. Regressions of seedling height against ethylene dose yielded  $R^2$  values of 0.011 and 0.018 with slopes of -0.419 ( $\pm 0.271$ ) and -1.099 ( $\pm 0.550$ ) with P values for 95% confidence intervals of 0.124 and 0.047 for pine and spruce, respectively.

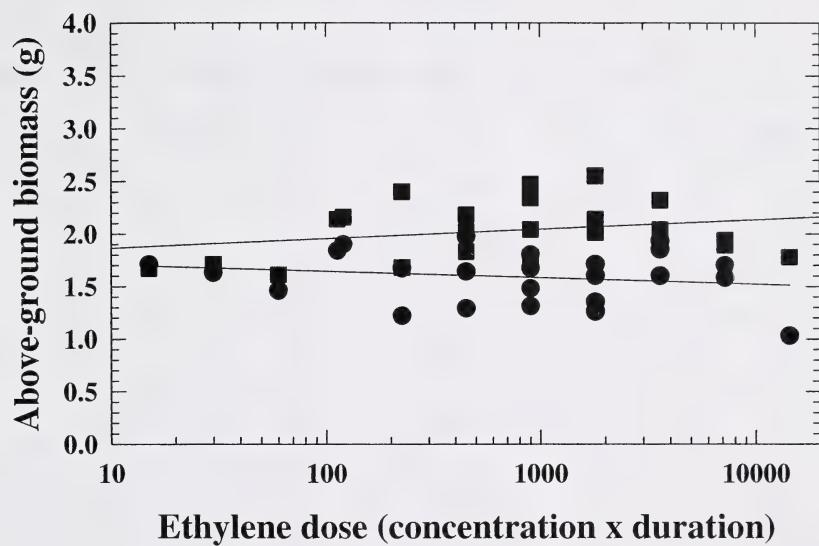


Figure 3.8. Dose (concentration x duration) response in seedling biomass of pine (squares) and spruce (circles) exposed to ethylene at the rapid growth phase. Dose is plotted on a log scale. Values are means where  $N = 8$  to 10. Regressions of seedling above-ground biomass against ethylene dose yielded  $R^2$  values of 0.018 and 0.008 with slopes of 0.098 ( $\pm 0.049$ ) and -0.060 ( $\pm 0.046$ ) with P values for 95% confidence intervals of 0.046 and 0.197 for pine and spruce, respectively.

Table 3.1. Vegetative and reproductive data for barley cv. Harrington. Plants were treated with ethylene at the spike emerging stage for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E.  
N = 5.

Length of Exp. (h)	Eth. conc. (ppb)	AG biomass (g)	Root biomass (g)	Number of heads	Weight of heads (g)
1.5	10	79.7 $\pm$ 5.5	6.6 $\pm$ 0.4	87 $\pm$ 7	37.7 $\pm$ 4.0
	75	70.5 $\pm$ 6.6	5.1 $\pm$ 0.2	83 $\pm$ 12	32.1 $\pm$ 6.4
	150	70.9 $\pm$ 5.6	5.7 $\pm$ 0.4	87 $\pm$ 6	35.2 $\pm$ 3.0
	300	80.7 $\pm$ 5.4	5.3 $\pm$ 1.1	91 $\pm$ 5	31.9 $\pm$ 5.0
	600	82.1 $\pm$ 2.8	6.8 $\pm$ 0.7	91 $\pm$ 5	40.3 $\pm$ 1.4
	1200	79.2 $\pm$ 6.9	6.7 $\pm$ 1.0	100 $\pm$ 11	33.6 $\pm$ 4.6
3	10	71.8 $\pm$ 3.2	4.7 $\pm$ 0.6	91 $\pm$ 8	48.2 $\pm$ 3.5
	75	68.7 $\pm$ 1.9	5.3 $\pm$ 0.4	84 $\pm$ 6	42.5 $\pm$ 5.5
	150	68.1 $\pm$ 5.4	4.9 $\pm$ 0.4	89 $\pm$ 3	41.7 $\pm$ 2.0
	300	77.0 $\pm$ 9.2	7.0 $\pm$ 1.3	92 $\pm$ 12	40.6 $\pm$ 6.8
	600	69.5 $\pm$ 8.6	6.2 $\pm$ 1.5	81 $\pm$ 10	39.2 $\pm$ 5.4
	1200	77.9 $\pm$ 5.1	5.9 $\pm$ 0.4	95 $\pm$ 10	41.6 $\pm$ 6.4

Table 3.1 continued ...

Length of Exp. (h)	Eth. conc. (ppb)	AG biomass (g)	Root biomass (g)	Number of heads	Weight of heads (g)
6	10	85.6 ± 4.8	7.6 ± 0.7	106 ± 9	44.8 ± 3.2
	75	74.3 ± 4.7	7.5 ± 0.8	89 ± 4	49.3 ± 2.6
	150	61.6 ± 2.9	5.1 ± 0.5	73 ± 7	35.9 ± 2.0
	300	79.4 ± 4.3	7.0 ± 0.5	81 ± 6	45.5 ± 2.6
	600	74.4 ± 4.8	6.6 ± 0.8	83 ± 8	46.1 ± 3.0
	1200	70.7 ± 4.5	5.9 ± 0.8	75 ± 7	37.5 ± 3.1
12	10	78.5 ± 6.1	4.5 ± 0.6	79 ± 13	29.2 ± 2.7
	75	79.8 ± 7.7	4.4 ± 0.7	86 ± 10	41.4 ± 4.7
	150	79.4 ± 7.0	5.0 ± 0.5	79 ± 3	43.0 ± 4.1
	300	62.3 ± 6.1	4.2 ± 0.5	87 ± 11	30.7 ± 2.3
	600	72.0 ± 8.5	4.0 ± 0.5	90 ± 6	30.1 ± 1.4
	1200	75.5 ± 4.3	4.5 ± 0.4	93 ± 8	34.5 ± 3.2

Table 3.2. Reproductive data for barley cv. Harrington. Plants were treated with ethylene at the spike emerging stage for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E. N = 5.

Length of Exp. (h)	Eth. conc. (ppb)	Number of seeds	Weight of seeds (g)	Weight per 1000 seeds (g)
1.5	10	387 $\pm$ 48	16.0 $\pm$ 2.4	40.8 $\pm$ 1.4
	75	346 $\pm$ 53	13.6 $\pm$ 2.7	37.6 $\pm$ 3.3
	150	348 $\pm$ 54	13.8 $\pm$ 2.1	39.9 $\pm$ 1.3
	300	314 $\pm$ 57	12.2 $\pm$ 2.5	38.0 $\pm$ 1.1
	600	370 $\pm$ 42	16.1 $\pm$ 2.1	43.1 $\pm$ 1.0
	1200	286 $\pm$ 26	10.6 $\pm$ 1.1	39.1 $\pm$ 2.2
3	10	543 $\pm$ 64	23.2 $\pm$ 3.0	42.5 $\pm$ 1.1
	75	535 $\pm$ 97	23.0 $\pm$ 4.8	41.8 $\pm$ 1.8
	150	550 $\pm$ 21	21.9 $\pm$ 0.5	40.0 $\pm$ 1.4
	300	510 $\pm$ 76	21.0 $\pm$ 4.0	40.1 $\pm$ 2.3
	600	480 $\pm$ 47	20.4 $\pm$ 2.5	42.0 $\pm$ 1.8
	1200	463 $\pm$ 94	20.3 $\pm$ 4.6	42.7 $\pm$ 1.7

Table 3.2 continued ...

Length of Exp. (h)	Eth. conc. (ppb)	Number of seeds	Weight of seeds (g)	Weight per 1000 seeds (g)
6	10	406 ± 49	18.1 ± 2.2	44.5 ± 1.0
	75	570 ± 54	23.8 ± 2.4	41.7 ± 1.5
	150	495 ± 56	19.7 ± 2.4	39.7 ± 0.9
	300	551 ± 62	23.6 ± 2.4	43.2 ± 1.4
	600	559 ± 46	24.6 ± 1.7	44.2 ± 1.1
	1200	452 ± 41	18.3 ± 2.3	40.1 ± 1.8
12	10	324 ± 51	13.0 ± 2.1	40.1 ± 0.4
	75	432 ± 51	18.7 ± 2.4	43.0 ± 1.2
	150	500 ± 68	21.5 ± 2.6	43.4 ± 1.4
	300	300 ± 34	11.6 ± 1.4	38.7 ± 1.2
	600	270 ± 60	10.8 ± 2.1	41.4 ± 2.3
	1200	309 ± 35	12.9 ± 1.4	41.9 ± 1.6

Table 3.3. Seed quality data for barley cv. Harrington. Plants were treated with ethylene at the spike emerging stage for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E. N = 5.

Length of Exp. (h)	Eth. conc. (ppb)	Calories Per Gram DW	% Protein per DW	% Calcium per DW	% Phosphorus per DW
1.5	10	4438 $\pm$ 8	22.7 $\pm$ 0.9	0.13 $\pm$ 0.00	0.60 $\pm$ 0.01
	75	4465 $\pm$ 29	23.5 $\pm$ 2.0	0.14 $\pm$ 0.01	0.59 $\pm$ 0.03
	150	4461 $\pm$ 15	23.4 $\pm$ 0.4	0.13 $\pm$ 0.01	0.60 $\pm$ 0.01
	300	4446 $\pm$ 17	23.5 $\pm$ 1.1	0.13 $\pm$ 0.00	0.58 $\pm$ 0.01
	600	4411 $\pm$ 8	21.4 $\pm$ 0.4	0.13 $\pm$ 0.00	0.58 $\pm$ 0.01
	1200	4445 $\pm$ 16	24.3 $\pm$ 1.2	0.13 $\pm$ 0.01	0.58 $\pm$ 0.00
3	10	4377 $\pm$ 17	22.3 $\pm$ 0.8	0.13 $\pm$ 0.00	0.52 $\pm$ 0.01
	75	4412 $\pm$ 13	22.8 $\pm$ 1.0	0.13 $\pm$ 0.00	0.55 $\pm$ 0.01
	150	4407 $\pm$ 21	22.3 $\pm$ 1.0	0.12 $\pm$ 0.00	0.54 $\pm$ 0.01
	300	4439 $\pm$ 18	23.2 $\pm$ 1.3	0.13 $\pm$ 0.00	0.54 $\pm$ 0.01
	600	4409 $\pm$ 14	21.8 $\pm$ 0.6	0.13 $\pm$ 0.00	0.52 $\pm$ 0.02
	1200	4409 $\pm$ 30	23.7 $\pm$ 2.5	0.13 $\pm$ 0.00	0.53 $\pm$ 0.02

Table 3.3 continued...

<b>Length of Exp. (h)</b>	<b>Eth. conc. (ppb)</b>	<b>Calories Per Gram DW</b>	<b>% Protein per DW</b>	<b>% Calcium per DW</b>	<b>% Phosphorus per DW</b>
<b>6</b>	<b>10</b>	4341 ± 2	21.0 ± 0.1	0.12 ± 0.00	0.49 ± 0.01
	<b>75</b>	4366 ± 15	21.9 ± 1.4	0.12 ± 0.00	0.49 ± 0.01
	<b>150</b>	4381 ± 11	24.5 ± 0.3	0.13 ± 0.00	0.49 ± 0.01
	<b>300</b>	4372 ± 25	21.7 ± 0.5	0.13 ± 0.00	0.47 ± 0.01
	<b>600</b>	4352 ± 11	22.4 ± 0.5	0.13 ± 0.00	0.45 ± 0.01
	<b>1200</b>	4384 ± 6	23.8 ± 0.5	0.12 ± 0.00	0.47 ± 0.02
<b>12</b>	<b>10</b>	4421 ± 8	24.3 ± 0.7	0.13 ± 0.00	0.57 ± 0.02
	<b>75</b>	4361 ± 12	22.7 ± 1.2	0.13 ± 0.00	0.54 ± 0.01
	<b>150</b>	4387 ± 18	21.5 ± 0.9	0.13 ± 0.00	0.53 ± 0.01
	<b>300</b>	4417 ± 9	23.5 ± 0.6	0.13 ± 0.00	0.57 ± 0.02
	<b>600</b>	4417 ± 11	23.2 ± 0.8	0.13 ± 0.01	0.57 ± 0.01
	<b>1200</b>	4400 ± 18	23.3 ± 1.3	0.13 ± 0.01	0.56 ± 0.02

Table 3.4. Vegetative and reproductive parameters for field pea cv. Carrera. Plants were treated with ethylene at the flat pod stage for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E. N = 5.

Length of exp. (h)	Eth. conc. (ppb)	AG biomass (g)	Number of yellow pods	Number of green pods	Total # of pods	Total weight of pods (g)
1.5	10	9.0 $\pm$ 0.9	8 $\pm$ 1	4 $\pm$ 1	12 $\pm$ 0	19.9 $\pm$ 1.6
	75	9.1 $\pm$ 0.3	10 $\pm$ 1	3 $\pm$ 1	13 $\pm$ 1	19.1 $\pm$ 0.7
	150	7.8 $\pm$ 0.5	9 $\pm$ 1	4 $\pm$ 2	13 $\pm$ 2	16.6 $\pm$ 1.5
	300	8.8 $\pm$ 0.6	10 $\pm$ 1	3 $\pm$ 1	13 $\pm$ 1	19.4 $\pm$ 1.6
	600	9.5 $\pm$ 1.6	8 $\pm$ 1	5 $\pm$ 2	13 $\pm$ 2	20.0 $\pm$ 1.7
	1200	8.1 $\pm$ 0.4	8 $\pm$ 1	3 $\pm$ 1	11 $\pm$ 1	18.4 $\pm$ 0.6
3	10	9.4 $\pm$ 0.4	8 $\pm$ 1	7 $\pm$ 2	15 $\pm$ 1	23.6 $\pm$ 1.2
	75	9.8 $\pm$ 0.3	13 $\pm$ 2	6 $\pm$ 1	18 $\pm$ 1	26.4 $\pm$ 0.9
	150	9.7 $\pm$ 1.0	10 $\pm$ 1	6 $\pm$ 2	16 $\pm$ 1	21.0 $\pm$ 0.9
	300	9.3 $\pm$ 0.7	6 $\pm$ 2	9 $\pm$ 2	15 $\pm$ 1	21.8 $\pm$ 1.3
	600	9.0 $\pm$ 0.3	10 $\pm$ 0	8 $\pm$ 2	18 $\pm$ 2	25.3 $\pm$ 1.4
	1200	9.6 $\pm$ 0.8	6 $\pm$ 1	9 $\pm$ 2	15 $\pm$ 1	22.4 $\pm$ 2.2

Table 3.4 continued ...

Length of exp. (h)	Eth. conc. (ppb)	AG biomass (g)	Number of yellow pods	Number of green pods	Total # of pods	Total weight of pods (g)
6	10	6.3 ± 1.0	8 ± 1	3 ± 2	11 ± 1	14.1 ± 1.4
	75	6.6 ± 0.4	7 ± 1	4 ± 2	11 ± 1	13.0 ± 1.3
	150	6.2 ± 0.4	9 ± 1	1 ± 1	10 ± 0	12.0 ± 0.8
	300	5.9 ± 0.5	11 ± 2	1 ± 1	12 ± 1	13.9 ± 1.0
	600	6.1 ± 0.2	8 ± 0	3 ± 1	11 ± 1	12.8 ± 1.2
	1200	6.1 ± 0.1	7 ± 1	4 ± 1	11 ± 1	13.1 ± 1.4
12	10	9.8 ± 0.4	9 ± 1	3 ± 1	12 ± 1	15.7 ± 1.0
	75	10.1 ± 1.1	9 ± 0	2 ± 1	11 ± 1	14.2 ± 0.6
	150	8.8 ± 1.3	9 ± 1	2 ± 1	11 ± 2	15.9 ± 0.9
	300	10.2 ± 0.5	9 ± 0	3 ± 1	12 ± 1	19.3 ± 0.3
	600	10.4 ± 0.8	9 ± 1	2 ± 1	11 ± 1	15.8 ± 1.9
	1200	12.1 ± 0.6	9 ± 1	4 ± 1	13 ± 1	15.8 ± 1.7

Table 3.5. Reproductive data for field pea cv. Carrera. Plants were treated with ethylene at the flat pod stage for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E. N = 5.

Length of exp. (h)	Eth. conc. (ppb)	Number of seeds	Weight of seeds (g)	Weight per 1000 seeds (g)
1.5	10	55 $\pm$ 6	16.3 $\pm$ 1.8	300.6 $\pm$ 13.8
	75	58 $\pm$ 1	16.7 $\pm$ 0.7	287.8 $\pm$ 6.7
	150	54 $\pm$ 4	14.0 $\pm$ 1.2	261.3 $\pm$ 13.3
	300	59 $\pm$ 4	16.6 $\pm$ 1.4	282.5 $\pm$ 15.0
	600	54 $\pm$ 5	17.1 $\pm$ 1.5	320.0 $\pm$ 2.6
	1200	53 $\pm$ 2	16.1 $\pm$ 0.5	303.2 $\pm$ 8.6
3	10	66 $\pm$ 4	20.0 $\pm$ 1.1	304.2 $\pm$ 7.9
	75	77 $\pm$ 3	22.8 $\pm$ 0.9	290.1 $\pm$ 12.6
	150	61 $\pm$ 1	17.9 $\pm$ 0.8	291.1 $\pm$ 11.7
	300	60 $\pm$ 4	18.0 $\pm$ 1.2	288.9 $\pm$ 16.6
	600	72 $\pm$ 4	21.5 $\pm$ 1.2	298.2 $\pm$ 5.3
	1200	65 $\pm$ 3	19.0 $\pm$ 1.9	291.3 $\pm$ 17.6

Table 3.5 continued ...

Length of exp. (h)	Eth. conc. (ppb)	Number of seeds	Weight of seeds (g)	Weight per 1000 seeds (g)
6	10	38 ± 2	12.1 ± 1.2	316.3 ± 20.8
	75	39 ± 3	11.2 ± 1.3	287.3 ± 21.1
	150	36 ± 2	10.4 ± 0.8	290.4 ± 11.8
	300	41 ± 4	12.1 ± 0.9	299.5 ± 15.3
	600	35 ± 2	10.8 ± 1.0	310.9 ± 11.3
	1200	36 ± 3	11.4 ± 1.2	314.4 ± 14.3
12	10	43 ± 3	13.5 ± 0.9	315.4 ± 5.6
	75	41 ± 3	12.1 ± 0.6	302.4 ± 16.7
	150	42 ± 2	13.5 ± 0.7	318.4 ± 3.4
	300	49 ± 3	15.5 ± 1.2	317.2 ± 5.1
	600	44 ± 7	13.6 ± 1.6	311.4 ± 11.5
	1200	43 ± 5	13.5 ± 1.4	317.9 ± 5.7

Table 3.6. Seed quality data for field pea cv. Carrera. Plants were treated with ethylene at the flat pod stage for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E. N = 5.

Length of exp. (h)	Eth. conc. (ppb)	Calories per gram DW	% Protein per DW	% Calcium per DW	% Phosphorus per DW
1.5	10	4226 $\pm$ 10	28.9 $\pm$ 0.9	0.63 $\pm$ 0.01	0.17 $\pm$ 0.01
	75	4215 $\pm$ 7	27.5 $\pm$ 0.5	0.62 $\pm$ 0.01	0.16 $\pm$ 0.01
	150	4192 $\pm$ 11	30.1 $\pm$ 0.4	0.63 $\pm$ 0.01	0.23 $\pm$ 0.02
	300	4219 $\pm$ 13	28.1 $\pm$ 0.7	0.63 $\pm$ 0.01	0.15 $\pm$ 0.01
	600	4195 $\pm$ 4	28.5 $\pm$ 0.7	0.63 $\pm$ 0.01	0.15 $\pm$ 0.01
	1200	4187 $\pm$ 7	28.8 $\pm$ 0.3	0.65 $\pm$ 0.01	0.17 $\pm$ 0.02
3	10	4167 $\pm$ 5	27.6 $\pm$ 0.3	0.62 $\pm$ 0.01	0.18 $\pm$ 0.02
	75	4171 $\pm$ 5	27.7 $\pm$ 0.7	0.60 $\pm$ 0.02	0.18 $\pm$ 0.01
	150	4172 $\pm$ 6	29.1 $\pm$ 0.9	0.64 $\pm$ 0.01	0.20 $\pm$ 0.02
	300	4171 $\pm$ 7	28.3 $\pm$ 0.6	0.65 $\pm$ 0.00	0.19 $\pm$ 0.01
	600	4167 $\pm$ 7	28.3 $\pm$ 0.5	0.64 $\pm$ 0.01	0.18 $\pm$ 0.02
	1200	4167 $\pm$ 5	27.9 $\pm$ 0.4	0.63 $\pm$ 0.01	0.17 $\pm$ 0.01

Table 3.6 continued ...

Length of exp. (h)	Eth. conc. (ppb)	Calories per gram DW	% Protein per DW	% Calcium per DW	% Phosphorus per DW
6	10	4176 ± 8	29.4 ± 0.7	0.65 ± 0.01	0.23 ± 0.02
	75	4182 ± 10	28.9 ± 1.3	0.64 ± 0.01	0.19 ± 0.01
	150	4178 ± 10	28.6 ± 0.7	0.63 ± 0.01	0.19 ± 0.02
	300	4167 ± 8	30.3 ± 1.0	0.64 ± 0.01	0.25 ± 0.04
	600	4182 ± 4	30.5 ± 0.4	0.67 ± 0.01	0.20 ± 0.02
	1200	4186 ± 11	28.5 ± 0.3	0.64 ± 0.01	0.21 ± 0.02
12	10	4210 ± 7	29.9 ± 0.5	0.64 ± 0.01	0.21 ± 0.02
	75	4217 ± 11	30.8 ± 0.8	0.66 ± 0.01	0.18 ± 0.02
	150	4201 ± 5	30.4 ± 0.4	0.67 ± 0.01	0.16 ± 0.01
	300	4208 ± 5	29.2 ± 0.6	0.65 ± 0.01	0.15 ± 0.01
	600	4210 ± 6	30.3 ± 1.0	0.66 ± 0.02	0.23 ± 0.02
	1200	4224 ± 5	30.5 ± 0.4	0.66 ± 0.01	0.22 ± 0.01

Table 3.7. Vegetative and reproductive data for canola cv. Quantum. Plants were treated with ethylene at the many flowers stage for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E. N = 5.

Length of Exp. (h)	Eth. conc. (ppb)	AG biomass (g)	Number of seeds	Weight of seeds (g)	Weight per 1000 seeds (g)
1.5	10	111.4 $\pm$ 7.4	7383 $\pm$ 487	22.3 $\pm$ 2.1	3.01 $\pm$ 0.13
	75	108.9 $\pm$ 16.9	7552 $\pm$ 945	25.2 $\pm$ 3.0	3.35 $\pm$ 0.09
	150	125.8 $\pm$ 18.3	6493 $\pm$ 1067	21.5 $\pm$ 4.0	3.27 $\pm$ 0.14
	300	140.3 $\pm$ 6.5	8162 $\pm$ 1059	31.6 $\pm$ 3.4	4.03 $\pm$ 0.57
	600	127.7 $\pm$ 8.2	8124 $\pm$ 587	25.8 $\pm$ 2.1	3.17 $\pm$ 0.10
	1200	135.2 $\pm$ 19.9	7925 $\pm$ 1072	26.4 $\pm$ 3.5	3.35 $\pm$ 0.13
3	10	123.0 $\pm$ 16.1	9756 $\pm$ 2365	25.6 $\pm$ 5.8	2.72 $\pm$ 0.15
	75	123.8 $\pm$ 7.9	8349 $\pm$ 871	23.3 $\pm$ 3.1	2.74 $\pm$ 0.24
	150	119.6 $\pm$ 13.9	9320 $\pm$ 880	27.7 $\pm$ 2.8	2.96 $\pm$ 0.10
	300	117.3 $\pm$ 20.0	9876 $\pm$ 1446	28.7 $\pm$ 4.9	2.84 $\pm$ 0.12
	600	103.9 $\pm$ 10.2	7264 $\pm$ 1013	20.4 $\pm$ 2.2	2.89 $\pm$ 0.18
	1200	103.1 $\pm$ 8.9	7581 $\pm$ 1538	18.7 $\pm$ 4.2	2.42 $\pm$ 0.10

Table 3.7 continued...

Length of Exp. (h)	Eth. conc. (ppb)	AG biomass (g)	Number of seeds	Weight of seeds (g)	Weight per 1000 seeds (g)
6	10	117.2 ± 12.8	7813 ± 752	27.9 ± 3.0	3.57 ± 0.16
	75	135.0 ± 4.1	9838 ± 831	32.7 ± 1.6	3.37 ± 0.17
	150	130.0 ± 20.7	7808 ± 805	28.6 ± 3.1	3.68 ± 0.13
	300	121.5 ± 7.6	8914 ± 584	28.8 ± 2.4	3.22 ± 0.09
	600	126.4 ± 19.0	10022 ± 1802	32.6 ± 5.1	3.34 ± 0.22
	1200	117.8 ± 6.0	9669 ± 923	33.1 ± 2.9	3.44 ± 0.16
12	10	130.5 ± 13.8	9906 ± 913	32.9 ± 4.4	3.29 ± 0.18
	75	119.0 ± 12.1	8916 ± 873	31.3 ± 3.3	3.51 ± 0.08
	150	148.3 ± 16.8	8617 ± 758	29.2 ± 1.9	3.43 ± 0.15
	300	142.2 ± 6.53	10636 ± 1693	36.5 ± 3.1	3.70 ± 0.54
	600	123.4 ± 3.9	9137 ± 971	27.2 ± 3.1	2.98 ± 0.13
	1200	117.4 ± 17.8	7492 ± 822	26.2 ± 3.1	3.50 ± 0.18

Table 3.8. Seed quality data for canola cv. Quantum. Plants were treated with ethylene at the many flowers stage for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E. N = 5.

Length of Exp. (h)	Eth. conc. (ppb)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	% Oil per DW
1.5	10	32.5 $\pm$ 1.4	0.30 $\pm$ 0.03	1.10 $\pm$ 0.06	33.7 $\pm$ 0.4
	75	33.2 $\pm$ 0.9	0.31 $\pm$ 0.00	1.07 $\pm$ 0.08	32.5 $\pm$ 0.6
	150	32.7 $\pm$ 0.7	0.31 $\pm$ 0.02	0.97 $\pm$ 0.05	31.0 $\pm$ 0.6
	300	32.3 $\pm$ 0.7	0.29 $\pm$ 0.01	0.99 $\pm$ 0.01	32.7 $\pm$ 1.0
	600	32.7 $\pm$ 0.4	0.30 $\pm$ 0.02	1.01 $\pm$ 0.03	31.5 $\pm$ 0.4
	1200	33.1 $\pm$ 0.3	0.28 $\pm$ 0.01	1.03 $\pm$ 0.01	32.3 $\pm$ 0.3
3	10	32.9 $\pm$ 0.7	0.34 $\pm$ 0.02	1.05 $\pm$ 0.04	31.2 $\pm$ 0.4
	75	34.0 $\pm$ 0.4	0.31 $\pm$ 0.03	1.10 $\pm$ 0.05	30.8 $\pm$ 0.8
	150	32.9 $\pm$ 0.3	0.31 $\pm$ 0.02	1.06 $\pm$ 0.04	31.5 $\pm$ 0.3
	300	31.8 $\pm$ 0.5	0.34 $\pm$ 0.02	1.04 $\pm$ 0.04	31.8 $\pm$ 0.4
	600	33.2 $\pm$ 0.6	0.37 $\pm$ 0.01	1.13 $\pm$ 0.06	31.3 $\pm$ 0.5
	1200	32.9 $\pm$ 0.4	0.37 $\pm$ 0.03	1.08 $\pm$ 0.03	30.3 $\pm$ 0.8

Table 3.8 continued...

Length of Exp. (h)	Eth. conc. (ppb)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	% Oil per DW
6	10	32.9 ± 0.2	0.32 ± 0.01	1.06 ± 0.03	34.2 ± 0.3
	75	33.1 ± 0.3	0.30 ± 0.01	1.06 ± 0.03	32.9 ± 0.3
	150	32.7 ± 0.4	0.29 ± 0.02	1.02 ± 0.01	33.5 ± 0.6
	300	33.3 ± 0.3	0.33 ± 0.02	1.15 ± 0.04	32.6 ± 0.2
	600	33.2 ± 0.5	0.29 ± 0.01	1.04 ± 0.03	33.1 ± 0.8
	1200	32.7 ± 0.6	0.31 ± 0.01	1.06 ± 0.03	33.8 ± 1.1
12	10	32.0 ± 0.6	0.31 ± 0.02	1.02 ± 0.04	33.0 ± 1.0
	75	31.6 ± 0.5	0.31 ± 0.01	1.00 ± 0.02	33.9 ± 0.4
	150	33.1 ± 0.7	0.31 ± 0.01	1.12 ± 0.06	32.89 ± 0.81
	300	32.6 ± 0.7	0.30 ± 0.01	1.02 ± 0.03	32.3 ± 0.3
	600	33.9 ± 0.3	0.30 ± 0.01	1.14 ± 0.03	31.4 ± 0.3
	1200	33.4 ± 0.7	0.30 ± 0.02	1.07 ± 0.05	31.9 ± 0.9

Table 3.9. Vegetative parameters of pine (*Pinus contorta*) seedlings. Seedlings in the rapid growth phase were treated with ethylene for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E. N = 9 or 10. N.D. = No data.

Length of Exp. (h)	Eth. conc. (ppb)	Stem thickness (mm)	Number of branches	Root biomass (g)
1.5	10	3.03 $\pm$ 0.14	5 $\pm$ 1	7.21 $\pm$ 0.10
	75	3.31 $\pm$ 0.15	5 $\pm$ 0	7.42 $\pm$ 0.08
	150	3.09 $\pm$ 0.14	4 $\pm$ 0	7.35 $\pm$ 0.13
	300	3.65 $\pm$ 0.13	5 $\pm$ 0	7.44 $\pm$ 0.07
	600	3.57 $\pm$ 0.14	6 $\pm$ 1	7.48 $\pm$ 0.10
	1200	N.D.	N.D.	N.D.
3	10	3.24 $\pm$ 0.12	4 $\pm$ 1	7.48 $\pm$ 0.12
	75	3.45 $\pm$ 0.27	5 $\pm$ 0	7.68 $\pm$ 0.28
	150	3.18 $\pm$ 0.12	5 $\pm$ 0	7.50 $\pm$ 0.12
	300	3.41 $\pm$ 0.16	5 $\pm$ 0	7.36 $\pm$ 0.19
	600	3.37 $\pm$ 0.15	5 $\pm$ 1	7.55 $\pm$ 0.13
	1200	3.32 $\pm$ 0.19	5 $\pm$ 1	7.73 $\pm$ 0.11

Table 3.9 continued...

Length of Exp. (h)	Eth. conc. (ppb)	Stem thickness (mm)	Number of branches	Root biomass (g)
6	10	3.18 ± 0.12	4 ± 1	7.47 ± 0.09
	75	3.20 ± 0.08	4 ± 1	7.07 ± 0.11
	150	3.34 ± 0.11	5 ± 1	7.72 ± 0.12
	300	3.23 ± 0.17	4 ± 0	7.69 ± 0.12
	600	3.48 ± 0.16	5 ± 1	7.53 ± 0.15
	1200	3.20 ± 0.10	5 ± 0	7.73 ± 0.11
12	10	3.19 ± 0.14	6 ± 1	7.43 ± 0.38
	75	3.59 ± 0.22	5 ± 1	7.15 ± 0.10
	150	3.31 ± 0.09	6 ± 0	7.58 ± 0.11
	300	3.20 ± 0.13	5 ± 0	7.21 ± 0.12
	600	3.23 ± 0.09	5 ± 0	7.47 ± 0.08
	1200	3.07 ± 0.11	5 ± 1	7.34 ± 0.14

Table 3.10. Vegetative parameters of spruce (*Picea glauca*) seedlings. Seedlings in the rapid growth phase were treated with ethylene for 1.5, 3, 6 or 12 hours. Values are means  $\pm$  S.E. N = 8 to 10.

Length of Exp. (h)	Eth. conc. (ppb)	Stem thickness (mm)	Number of branches	Root biomass (g)
1.5	10	2.97 $\pm$ 0.10	14 $\pm$ 3	6.31 $\pm$ 0.09
	75	2.90 $\pm$ 0.12	13 $\pm$ 2	6.21 $\pm$ 0.18
	150	2.91 $\pm$ 0.10	17 $\pm$ 1	6.05 $\pm$ 0.09
	300	3.19 $\pm$ 0.19	11 $\pm$ 1	8.73 $\pm$ 0.97
	600	2.92 $\pm$ 0.06	7 $\pm$ 1	6.20 $\pm$ 0.10
	1200	2.99 $\pm$ 0.12	6 $\pm$ 1	5.62 $\pm$ 0.13
3	10	3.20 $\pm$ 0.12	12 $\pm$ 2	6.76 $\pm$ 0.07
	75	2.84 $\pm$ 0.05	9 $\pm$ 1	6.30 $\pm$ 0.08
	150	2.93 $\pm$ 0.07	15 $\pm$ 2	6.61 $\pm$ 0.08
	300	3.16 $\pm$ 0.08	12 $\pm$ 2	6.61 $\pm$ 0.11
	600	2.97 $\pm$ 0.10	10 $\pm$ 2	6.30 $\pm$ 0.10
	1200	3.20 $\pm$ 0.09	12 $\pm$ 2	6.19 $\pm$ 0.09

Table 3.10 continued...

Length of Exp. (h)	Eth. conc. (ppb)	Stem thickness (mm)	Number of branches	Root biomass (g)
6	10	3.28 ± 0.17	8 ± 1	6.35 ± 0.14
	75	3.14 ± 0.19	9 ± 3	6.85 ± 0.15
	150	3.03 ± 0.25	8 ± 1	6.67 ± 0.08
	300	2.92 ± 0.24	15 ± 2	5.99 ± 0.20
	600	2.76 ± 0.11	8 ± 2	6.20 ± 0.10
	1200	2.90 ± 0.14	9 ± 2	5.87 ± 0.39
12	10	3.29 ± 0.12	15 ± 2	6.76 ± 0.08
	75	3.32 ± 0.12	12 ± 2	6.53 ± 0.09
	150	3.12 ± 0.17	9 ± 1	6.88 ± 0.10
	300	2.86 ± 0.20	12 ± 2	6.12 ± 0.09
	600	3.54 ± 0.54	9 ± 2	6.43 ± 0.08
	1200	2.76 ± 0.14	9 ± 3	7.31 ± 1.13

## **4. RESPONSES TO LONG-TERM EXPOSURE**

### **4.1 Introduction**

The purpose of these experiments was to use a series of long-term exposures of ethylene and varying concentrations to determine the concentration threshold at which long-term exposures to ethylene would cause significant effects on the parameters measured. In these experiments, long-term exposures were defined as the length of time spanning the first sensitive stage to the last sensitive stage for each crop studied. Sensitive stages were determined in previous experiments using ethephon applications at several growth stages (Archambault and Li 1999).

### **4.2 Exposure Regimes**

Plants were grown in a greenhouse until they reached the first ethylene-sensitive stage (Archambault and Li 1999) and then transferred to growth chambers one day prior to exposure to ethylene. All exposures were performed and verified as described in section 2.6. Plants were exposed to either 10 (control), 50, 100, 150, 250 or 400 ppb ethylene throughout the sensitive stages. These stages were flag leaf emerging to early anthesis (26 days) for barley, first buds visible to 50% of pods full (16 days) for field pea and many flowers open to pods full (31 days) for canola. Because effects on barley were seen at concentrations as low as 50 ppb, a second trial was conducted in which a 30 ppb treatment was substituted for the 400 ppb treatment and exposures were shortened to 14 days. In each case, plants were returned to the greenhouse one day following exposures and grown until harvest.

### **4.3 Results**

Long-term exposures of barley to ethylene at concentrations of up to 400 ppb for 26 days had no effect on above-ground biomass (Fig. 4.1), number of tillers and stem thickness (Table 4.1). Root biomass increased by up to 123% (Fig. 4.2) at 400 ppb while the number of heads, weight of heads and seed number per pot decreased by up to 89, 98, 100%, respectively (Table 4.1). Seed yields decreased by 59% at 50 ppb and were nearly nil at 250 ppb and above (Fig. 4.3). Decreases in seed yield were not only due to lower seed numbers (Table 4.1) but also caused by a decrease of up to 75% in seed size (Table 4.2). While few seed quality data could be obtained because of low yields, exposures at 50 ppb had no significant effects on seed protein, calcium and phosphorus contents (Table 4.2).

Similarly to 26-day exposures, exposures of barley for 14 days had no effect on above-ground biomass (Fig. 4.5) and caused increases in root biomass of up to 46% (Fig. 4.6). A significant decrease in plant height was observed when plants were exposed to ethylene at 250 ppb and the number of tillers per pot increased by 202% at 400 ppb (Table 4.3). Differences in tillering effects between the 26-day and the 14-day exposures might suggest that the additional time between the end of treatments and plant maturity (harvest) allowed for the additional production of tillers in the 14-day exposures. Increased tillering of cereals in response to ethylene exposure has been reported in the literature (Foster et al. 1991, 1992; Taylor et al. 1991). Seed yields decreased by 63% at 30 ppb, by 72% at 50 ppb and were nearly nil at 100 ppb and above (Fig. 4.7). Seed yields in this experiment were many-fold lower than those obtained in the previous experiment due to an aphid infestation that occurred at the seed filling stages. Nevertheless, similarities of the patterns and relative quantities of yield losses between the two experiments suggest that effects of ethylene exposure and those of aphid attack were independent. Number of heads, weight of heads and number of seeds decreased by 88, 97 and 97%, respectively (Table 4.3). Again, reductions in yields were partially due to decreases in seed size (Table 4.3). Small seed samples did not allow for seed quality analyses in this experiment.

In both trials nicotine fumigation was used to control aphids. In the 26-day exposure experiment, nicotine treatment occurred one week prior to harvest. It is unlikely that ethylene released during nicotine treatment had any effect on the plants. In the 14-day exposure experiment, nicotine treatment was used both before and after exposure to ethylene. The first nicotine treatment was applied well in advance of the ethylene treatment; therefore, multiple exposure effects seem unlikely. The second nicotine treatment was 14 days prior to harvest, therefore, again, it is unlikely the nicotine treatment had any effect on plants. Normalizing the data from both trials shows that the response was virtually identical in terms of yield suppression as a function of ethylene concentration (data not show). This suggests that neither of these experiments were affected by nicotine treatment.

Exposures of field pea to ethylene at concentrations of up to 400 ppb for 16 days caused increases in above-ground biomass of up to 223% when plants were harvested when the control

plants were mature (Fig. 4.8) and of up to 197% when treated plants were harvested at maturity (Fig. 4.9). In plants harvested when controls reached maturity, root biomass was much as doubled in plants treated with 400 ppb ethylene (Fig. 4.10). Root biomass reached 227% of controls when plants treated with 400 ppb ethylene were grown to maturity (data not shown). Plant heights increased by up to 19% at 400 ppb whereas stem thickness remained unchanged (Table 4.4). Seed yields of plants harvested when control plants were mature decreased by up to 73% at 400 ppb ethylene (Fig. 4.11) but no effects on seed yields were observed when all plants were grown to maturity (Fig. 4.12). Observed decreases in seed yields appeared to be caused solely by decreased seed size (Table 4.5) since seed numbers were mostly unaffected by ethylene treatment (Table 4.4). Ethylene treatments tended to cause increases in seed calcium and phosphorus but these effects were not always statistically significant (Table 4.5). No effects on seed protein and caloric contents were observed (Table 4.5).

Exposures of canola to ethylene at concentrations of up to 400 ppb for 31 days caused decreases in above-ground biomass of up to 18% (Fig. 4.13) while no effects on root biomass were observed (Fig. 4.14). Plant heights were reduced by as much as 40% while stem thickness was unaffected (Table 4.6). This is in contrast to the effects of long-term exposures to ethylene that were observed in barley and field pea. For example, plant heights were unaffected in barley and increased in field pea. This illustrates species-specific effects of ethylene on vegetative characteristics. Seed yields decreased by up to 77% (Fig. 4.15) and this decrease was caused primarily by decreases in seed number as opposed to seed size (Table 4.6). Seed protein, calcium, phosphorus and oil contents were unaffected by ethylene treatment (Table 4.7).

#### 4.4 Discussion

Long-term exposures caused a variety of symptoms in the crops studied (Photographs are shown in Appendix II) and more pronounced effects on vegetative characters in field pea than on barley or canola. Similar to the effects of ethylene on kidney bean (Abeles and Heggestad 1973), symptoms of ethylene in field pea included, broken tendril, tendril curl, leaf curl, branching, thickened stem, broken stem, chlorosis and necrosis. In barley, we observed reduced plant heights, increased tillering and floret abortion, consistent with the results of Abeles and Heggestad (1973) for spring wheat. Aborted florets, increased tillering, chlorosis,

necrosis, smaller spikes and aborted seed production were also observed in barley. Ethylene caused reduced flower bud size, aborted flowers, reduced flower size, darker leaves and reduced plant size, in canola. Ethylene effects may cause vegetative effects, reproductive effects, or both. Reductions in seed yield in canola have been found in the absence of vegetative damage (Reid and Watson 1985).

Negative effects on seed yields were found in the three species studied. Only field pea demonstrated significant recovery after exposures to ethylene. The delay in plant maturity observed in plants treated at 150 ppb ethylene and above was of the same length as the length of exposure, 16 days. The relatively short growing season required to grow field pea might allow for full yield recoveries in the field even after long-term exposures to ethylene at the sensitive stages. Reversibility of ethylene effects appears to depend on concentration and duration of exposure but also on the nature and extent of the effects and on the species in question. Flowering in field pea was delayed but not abolished in the present study. Legumes and curcurbits have been shown to senesce irreversibly while composites and monocots have been found to recover (Abeles et al. 1992). Increases in biomass as measured in field pea may be beneficial if plants are grown for silage or for green manuring. In barley, significant yield losses could occur in 14 days at concentrations as low as 30 ppb. Barley did not appear to have the yield recovery abilities of field pea. For the purpose of this study, yield losses were deemed more important than effects on vegetative characters. Therefore, the critical length of exposure that would cause significant yield losses was determined in subsequent experiments.

#### **4.5 Conclusions**

Several symptoms of ethylene on vegetative and reproductive characters were observed in the three species studied. Ethylene symptoms appeared earliest in field pea and symptoms were most noticeable. Significant reductions in yield were observed in plants exposed to above 100 ppb ethylene for 16 days. In barley, significant yield losses occurred in 14 days at concentrations of 30 ppb. In canola, yields were reduced significantly in 31 days at 50 ppb. Complete recovery of yield in field pea was observed as well as the recovery of vegetative growth. Barley and canola did not appear to have the recovery abilities of field pea.

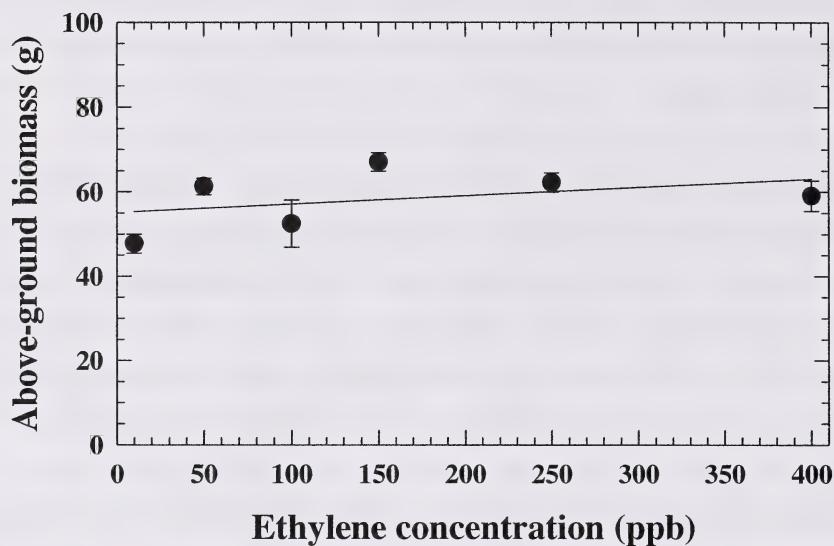


Figure 4.1. Effects of 26 days of exposure to ethylene at various concentrations on above-ground biomass in barley cv. Harrington. Plants were exposed to ethylene from the flag leaf emerging to the early anthesis stage. Values are means  $\pm$  S.E. N = 6.

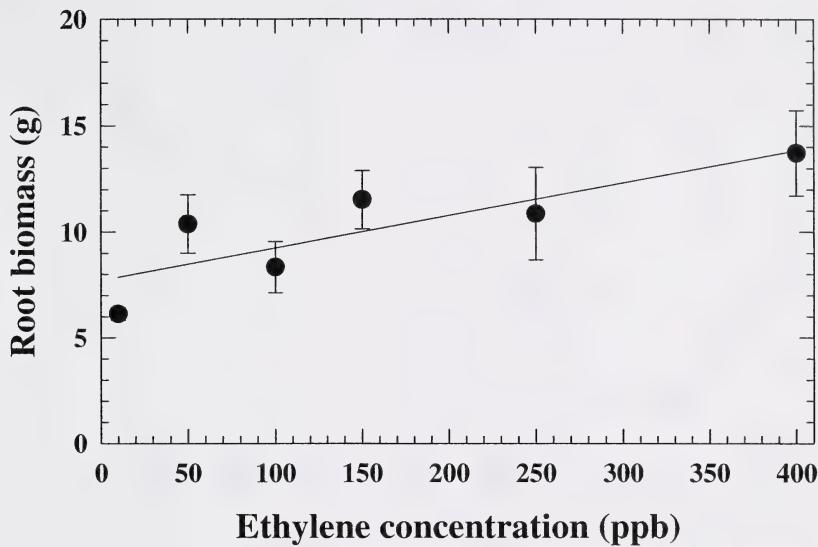


Figure 4.2. Effects of 26 days of exposure to ethylene at various concentrations on root biomass in barley cv. Harrington. Plants were exposed to ethylene from the flag leaf emerging to the early anthesis stage. Values are means  $\pm$  S.E. N = 6.

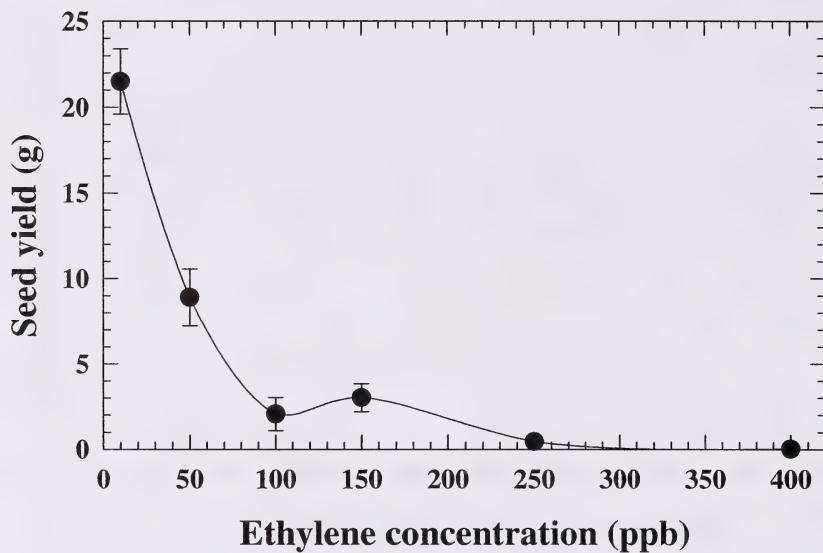


Figure 4.3. Effects of 26 days of exposure to ethylene at various concentrations on seed yield in barley cv. Harrington. Plants were exposed to ethylene from the flag leaf emerging to the early anthesis stage. Values are means  $\pm$  S.E. N = 6.

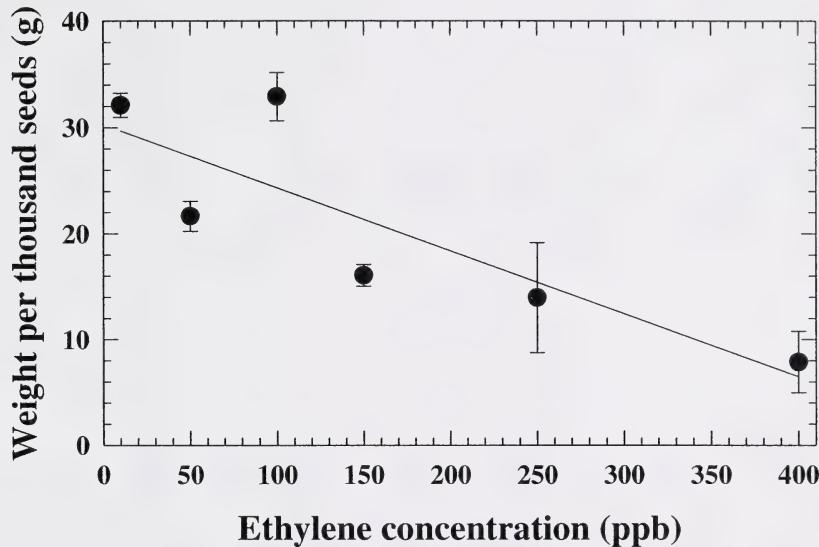


Figure 4.4. Effects of 26 days of exposure to ethylene at various concentrations on seed size in barley cv. Harrington. Plants were exposed to ethylene from the flag leaf emerging to the early anthesis stage. Values are means  $\pm$  S.E. N = 6.

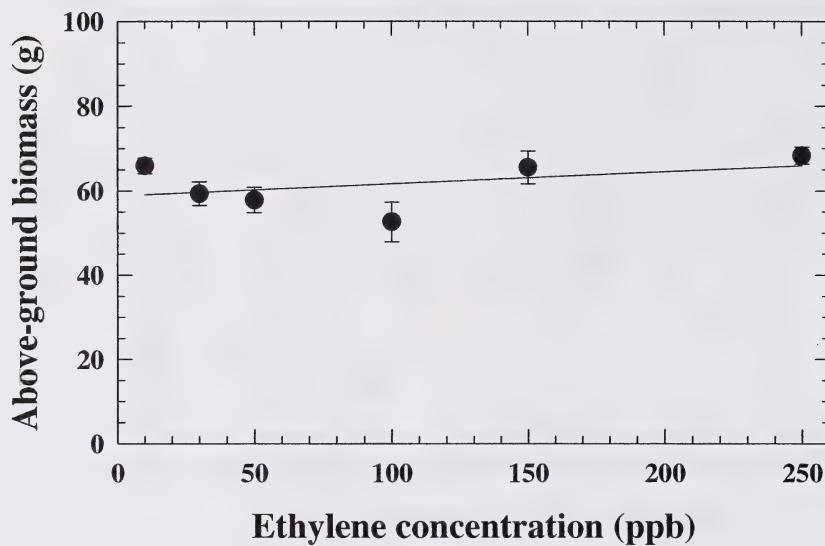


Figure 4.5. Effects of 14 days of exposure to ethylene at various concentrations on above-ground biomass in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. N = 6.

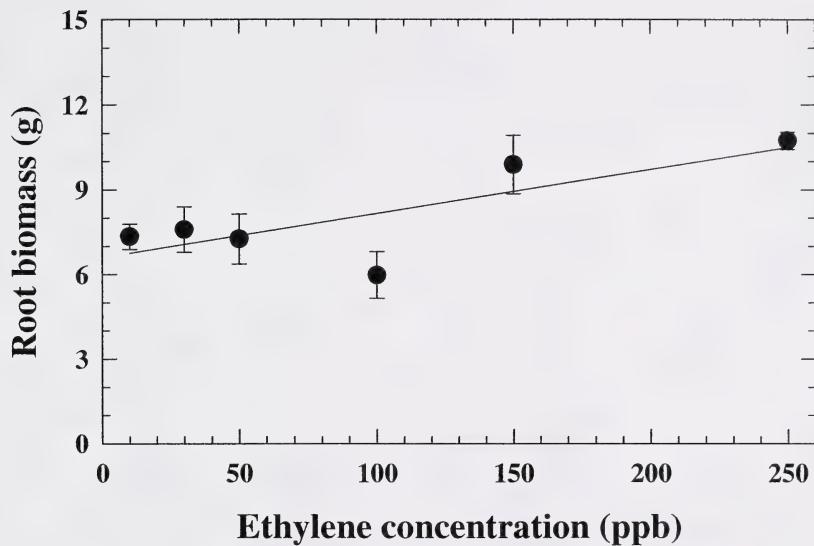


Figure 4.6. Effects of 14 days of exposure to ethylene at various concentrations on root biomass in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. N = 6.

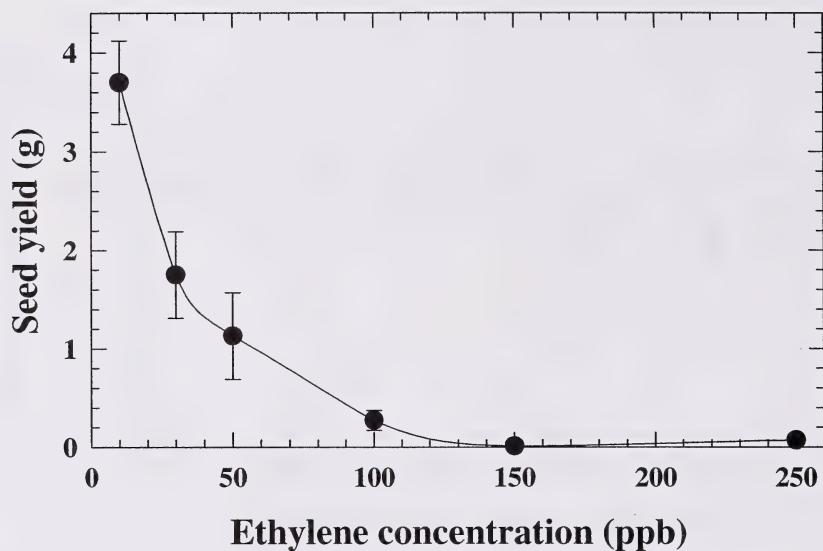


Figure 4.7. Effects of 14 days of exposure to ethylene at various concentrations on seed yield in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. N = 6.

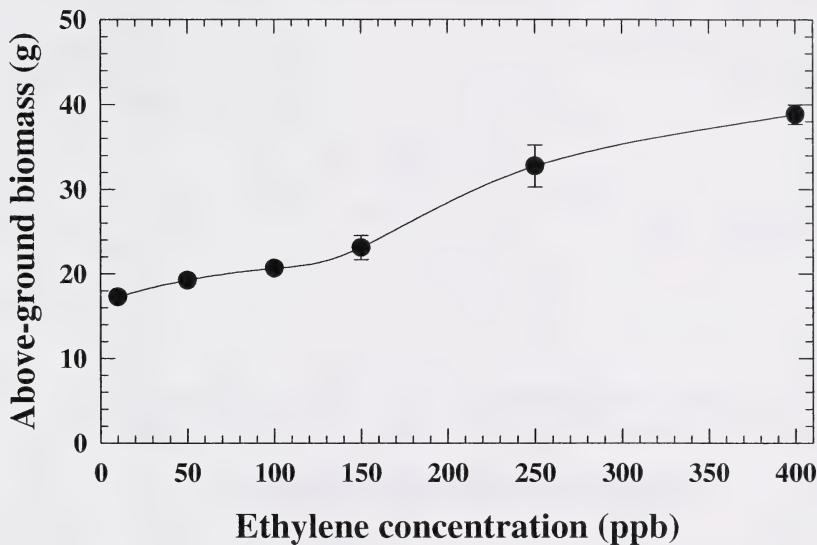


Figure 4.8. Effects of 16 days of exposure to ethylene at various concentrations on above-ground biomass in field pea cv. Carrera. Plants were exposed to ethylene from the first buds visible to the 50% pods full stage. Plants treated with 10, 50, 100 and 150 ppb reached maturity at the same time and were all harvested at maturity. Three replicates of each of the plants exposed to 250 and 400 ppb were also harvested at that time and the remaining three were grown to maturity (see Figure 4.9). The data for exposures at 250 and 400 ppb presented here are from plants harvested when the controls were mature. Values are means  $\pm$  S.E. N = 3 or 6.

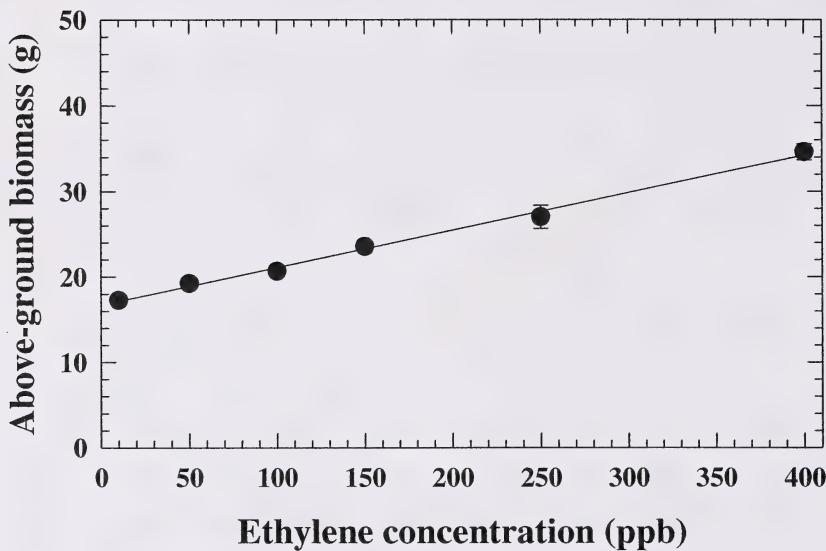


Figure 4.9. Effects of 16 days of exposure to ethylene at various concentrations on above-ground biomass in field pea cv. Carrera. Plants were exposed to ethylene from the first buds visible to the 50% pods full stage. Graph shows the same data as in Figure 4.8 for the 10, 50, 100 and 150 ppb treatments with three replicates from the 250 and 400 ppb treatments that were harvested at maturity. Values are means  $\pm$  S.E. N = 3 or 6.

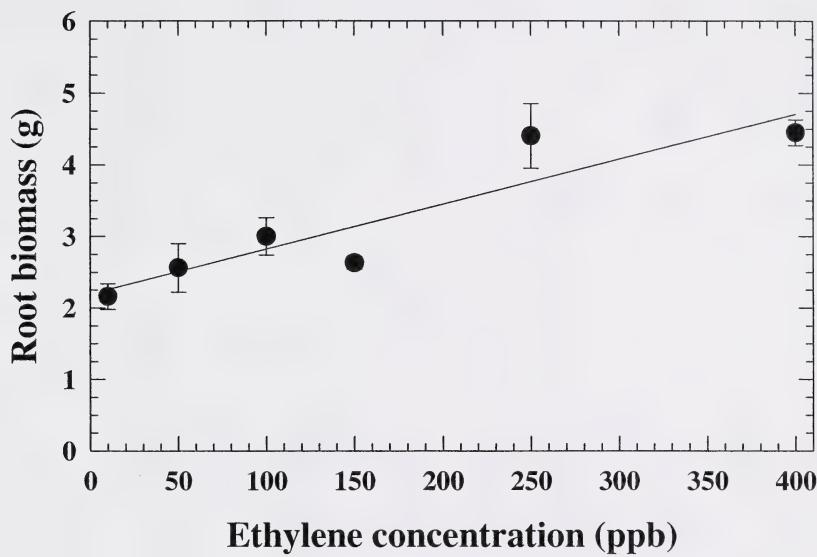


Figure 4.10. Effects of 16 days of exposure to ethylene at various concentrations on root biomass in field pea cv. Carrera. Plants treated with 10, 50, 100 and 150 ppb reached maturity at the same time and were all harvested at maturity. Three replicates of each of the plants exposed to 250 and 400 ppb were also harvested at that time and the remaining three were grown to maturity. The data for exposures at 250 and 400 ppb presented here are from plants harvested when the controls were mature. Values are means  $\pm$  S.E. N = 3 or 6.

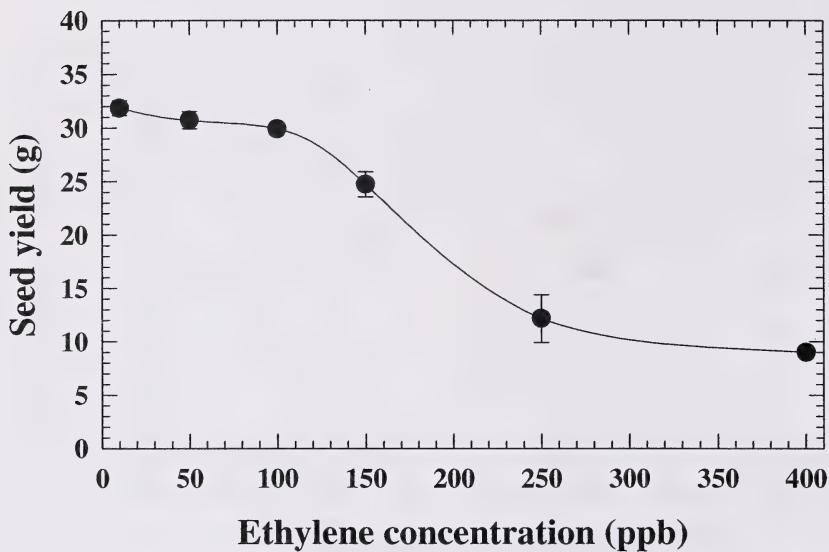


Figure 4.11. Effects of 16 days of exposure to ethylene at various concentrations on seed yield in field pea cv. Carrera. Plants were exposed to ethylene from the first buds visible to the 50% pods full stage. Plants treated with 10, 50, 100 and 150 ppb reached maturity at the same time and were all harvested at maturity. Three replicates of each of the plants exposed to 250 and 400 ppb were also harvested at that time and the remaining three were grown to maturity (see Figure 4.12). The data for exposures at 250 and 400 ppb presented here are from plants harvested when the controls were mature. Values are means  $\pm$  S.E. N = 3 or 6.

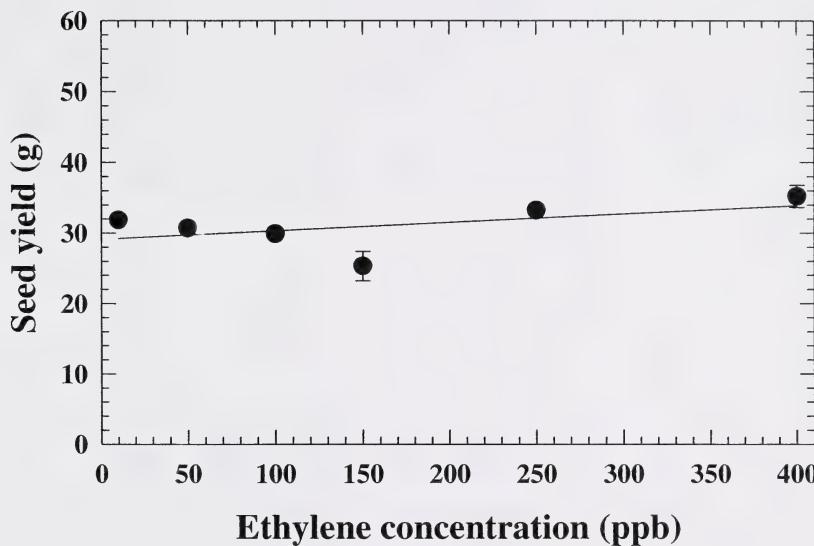


Figure 4.12. Effects of 16 days of exposure to ethylene at various concentrations on above-ground biomass in field pea cv. Carrera. Plants were exposed to ethylene from the first buds visible to the 50% pods full stage. Graph shows the same data as in Figure 4.11 for the 10, 50, 100 and 150 ppb treatments with three replicates from the 250 and 400 ppb treatments that were harvested at maturity. Values are means  $\pm$  S.E. N = 3 or 6.

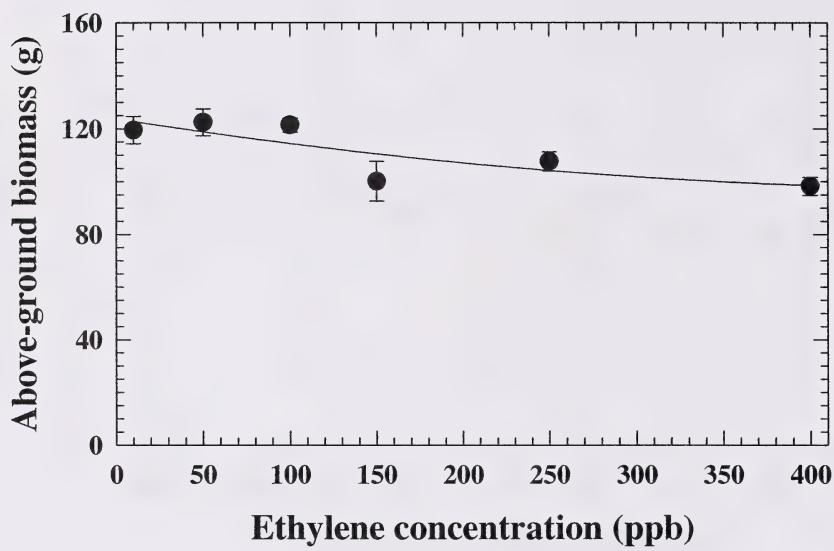


Figure 4.13. Effects of 31 days of exposure to ethylene at various concentrations on above-ground biomass in canola cv. Quantum. Plants were exposed to ethylene from the many flowers open stage to pods full stage. Values are means  $\pm$  S.E. N = 6.

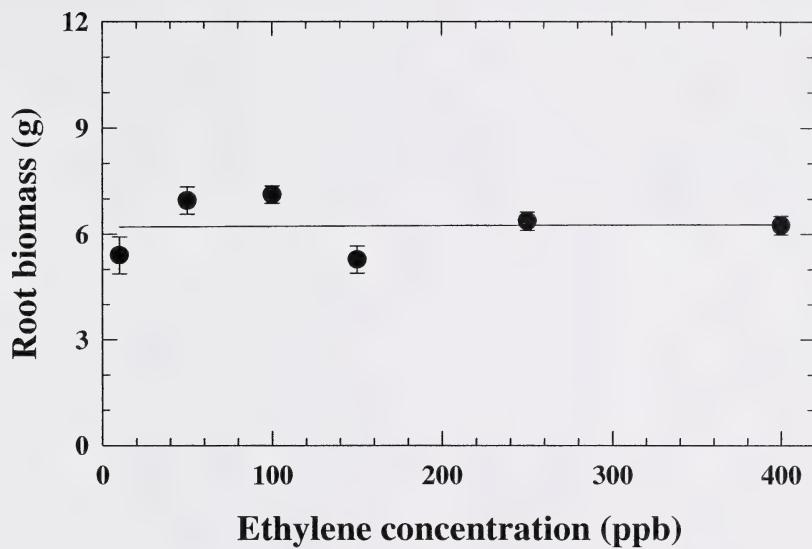


Figure 4.14. Effects of 31 days of exposure to ethylene at various concentrations on root biomass in canola cv. Quantum. Plants were exposed to ethylene from the many flowers open stage to pods full stage. Values are means  $\pm$  S.E. N = 6.

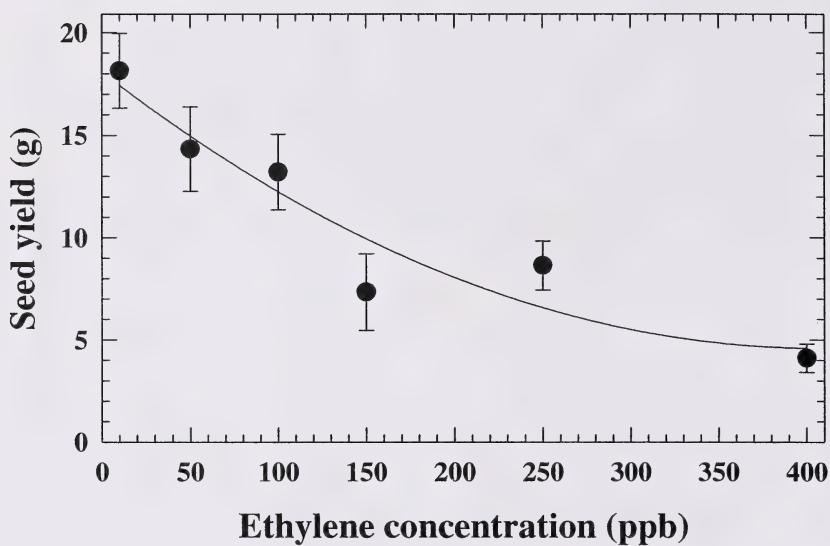


Figure 4.15. Effects of 31 days of exposure to ethylene at various concentrations on seed yield in canola cv. Quantum. Plants were exposed to ethylene from the many flowers open stage to pods full stage. Values are means  $\pm$  S.E. N = 6.

Table 4.1. Effects of 26 days of exposure to ethylene at various concentrations on vegetative and reproductive characters in barley cv. Harrington. Plants were exposed to ethylene from the flag leaf emerging to the early anthesis stage. Values are means  $\pm$  S.E. Plant height, N = 18; all others, N = 6.

Ethylene conc. (ppb)	Plant height (cm)	Number of tillers	Stem thickness (mm)	Number of heads	Weight of heads (g)	Number of seeds
10	75.7 $\pm$ 0.7	112 $\pm$ 4	4.2 $\pm$ 0.1	94 $\pm$ 6	34.7 $\pm$ 2.8	683 $\pm$ 82
50	68.9 $\pm$ 0.4	150 $\pm$ 18	4.1 $\pm$ 0.1	107 $\pm$ 5	19.9 $\pm$ 2.3	432 $\pm$ 88
100	69.7 $\pm$ 1.5	133 $\pm$ 23	4.2 $\pm$ 0.1	30 $\pm$ 14	4.2 $\pm$ 2.0	72 $\pm$ 41
150	70.2 $\pm$ 0.8	169 $\pm$ 17	4.4 $\pm$ 0.1	83 $\pm$ 10	9.9 $\pm$ 1.6	194 $\pm$ 53
250	69.2 $\pm$ 0.7	157 $\pm$ 11	4.0 $\pm$ 0.1	24 $\pm$ 9	2.1 $\pm$ 0.9	21 $\pm$ 12
400	68.1 $\pm$ 1.0	141 $\pm$ 20	3.9 $\pm$ 0.1	10 $\pm$ 3	0.6 $\pm$ 0.2	2 $\pm$ 1

Table 4.2. Effects of 26 days of exposure to ethylene at various concentrations on seed quality in barley cv. Harrington. Plants were exposed to ethylene from the flag leaf emerging to the early anthesis stage. Values are means  $\pm$  S.E. N = 3 to 6. I.S. = insufficient sample for analysis.

Ethylene conc. (ppb)	Weight per 1000 seeds (g)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	Calories per gram DW
10	32.1 $\pm$ 1.1	25.8 $\pm$ 0.7	0.17 $\pm$ 0.01	0.64 $\pm$ 0.01	4391 $\pm$ 5
50	21.7 $\pm$ 1.4	28.1 $\pm$ 0.6	0.17 $\pm$ 0.00	0.65 $\pm$ 0.02	4389 $\pm$ 17
100	32.9 $\pm$ 2.3	I.S.	I.S.	I.S.	4303 $\pm$ 29
150	16.1 $\pm$ 1.0	I.S.	I.S.	I.S.	4383 $\pm$ 1
250	13.9 $\pm$ 5.2	I.S.	I.S.	I.S.	4310 $\pm$ 11
400	7.9 $\pm$ 2.9	I.S.	I.S.	I.S.	I.S.

Table 4.3. Effects of 14 days of exposure to ethylene at various concentrations on vegetative and reproductive characters in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Plant height, N = 18; all others N = 6.

Ethylene conc. (ppb)	Plant height (cm)	Number of tillers	Number of heads	Weight of heads (g)	Number of seeds	Weight per 1000 seeds (g)
10	69.9 $\pm$ 1.2	145 $\pm$ 9	33 $\pm$ 3	8.6 $\pm$ 0.8	135 $\pm$ 15	27.7 $\pm$ 1.4
30	68.6 $\pm$ 1.0	145 $\pm$ 11	23 $\pm$ 3	5.4 $\pm$ 0.7	68 $\pm$ 14	24.7 $\pm$ 1.4
50	70.3 $\pm$ 1.7	133 $\pm$ 11	19 $\pm$ 2	3.7 $\pm$ 0.7	42 $\pm$ 10	24.6 $\pm$ 3.4
100	68.8 $\pm$ 1.0	155 $\pm$ 25	9 $\pm$ 2	1.0 $\pm$ 0.3	12 $\pm$ 4	16.1 $\pm$ 4.6
150	68.5 $\pm$ 1.3	265 $\pm$ 9	5 $\pm$ 1	0.3 $\pm$ 0.0	1 $\pm$ 1	3.6 $\pm$ 2.3
250	66.8 $\pm$ 0.9	293 $\pm$ 9	4 $\pm$ 1	0.3 $\pm$ 0.1	4 $\pm$ 2	10.0 $\pm$ 5.1

Table 4.4. Effects of 16 days of exposure to ethylene at various concentrations on vegetative and reproductive characters in field pea cv. Carrera. Plants were exposed to ethylene from the first buds visible stage to 50% of pods full stage. Values are means  $\pm$  S.E. N = 6.

Ethylene conc. (ppb)	Plant height (cm)	Stem thickness (mm)	Number of pods	Weight of pods (g)	Number of seeds
10	67.3 $\pm$ 1.8	2.08 $\pm$ 0.07	31 $\pm$ 1	36.5 $\pm$ 0.8	122 $\pm$ 3
50	67.4 $\pm$ 1.9	2.34 $\pm$ 0.15	34 $\pm$ 3	35.0 $\pm$ 1.0	125 $\pm$ 4
100	68.3 $\pm$ 1.6	1.63 $\pm$ 0.18	30 $\pm$ 1	34.4 $\pm$ 0.5	110 $\pm$ 2
150	71.1 $\pm$ 1.7	1.84 $\pm$ 0.14	24 $\pm$ 1	28.5 $\pm$ 1.4	87 $\pm$ 6
250	77.8 $\pm$ 2.7	2.01 $\pm$ 0.14	68 $\pm$ 11	22.3 $\pm$ 0.6	100 $\pm$ 12
400	80.2 $\pm$ 1.3	2.26 $\pm$ 0.11	62 $\pm$ 6	17.1 $\pm$ 0.5	144 $\pm$ 7

Table 4.5. Effects of 16 days of exposure to ethylene at various concentrations on seed quality in field pea cv. Carrera. Plants were exposed to ethylene from the first buds visible stage to 50% of pods full stage. Values are means  $\pm$  S.E. N = 3 to 6.

Ethylene conc. (ppb)	Weight per 1000 seeds (g)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	Calories per gram DW
10	261.3 $\pm$ 6.2	26.4 $\pm$ 1.9	0.18 $\pm$ 0.01	0.62 $\pm$ 0.02	4024 $\pm$ 7
50	247.1 $\pm$ 8.2	28.5 $\pm$ 0.2	0.16 $\pm$ 0.01	0.64 $\pm$ 0.00	4021 $\pm$ 10
100	271.2 $\pm$ 1.7	28.5 $\pm$ 0.2	0.14 $\pm$ 0.01	0.64 $\pm$ 0.00	4025 $\pm$ 8
150	286.2 $\pm$ 9.4	28.8 $\pm$ 0.4	0.20 $\pm$ 0.02	0.64 $\pm$ 0.01	4001 $\pm$ 7
250	121.2 $\pm$ 15.6	28.7 $\pm$ 0.2	0.14 $\pm$ 0.01	0.65 $\pm$ 0.01	4051 $\pm$ 26
400	62.9 $\pm$ 5.1	27.9 $\pm$ 0.7	0.13 $\pm$ 0.03	0.64 $\pm$ 0.00	4038 $\pm$ 9

Table 4.6. Effects of 31 days of exposure to ethylene at various concentrations on vegetative and reproductive characters in canola cv. Quantum. Plants were exposed to ethylene from the many flowers open stage to pods full stage. Height and thickness measurements were taken immediately following ethylene exposures. Values are means  $\pm$  S.E. Stem thickness, N = 24; others N = 6.

Ethylene conc. (ppb)	Plant height (cm)	Stem thickness (mm)	Seed number	Weight per 1000 seeds (g)
10	146.4 $\pm$ 3.1	10.3 $\pm$ 0.3	5906 $\pm$ 667	3.1 $\pm$ 0.1
50	128.2 $\pm$ 2.3	10.6 $\pm$ 0.3	4910 $\pm$ 751	3.0 $\pm$ 0.2
100	123.8 $\pm$ 0.9	12.2 $\pm$ 1.5	4362 $\pm$ 625	3.1 $\pm$ 0.2
150	118.6 $\pm$ 5.7	9.5 $\pm$ 0.3	2612 $\pm$ 697	2.9 $\pm$ 0.1
250	108.9 $\pm$ 1.6	10.9 $\pm$ 0.3	2989 $\pm$ 401	2.9 $\pm$ 0.
400	88.5 $\pm$ 4.5	11.2 $\pm$ 0.4	2348 $\pm$ 611	2.1 $\pm$ 0.3

Table 4.7. Effects of 31 days of exposure to ethylene at various concentrations on seed quality in canola cv. Quantum. Plants were exposed to ethylene from the many flowers open stage to pods full stage. Values are means  $\pm$  S.E. N = 6.

Ethylene conc. (ppb)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	% Oil content
10	36.9 $\pm$ 0.3	0.33 $\pm$ 0.02	1.14 $\pm$ 0.04	31.4 $\pm$ 0.5
50	36.0 $\pm$ 0.3	0.34 $\pm$ 0.01	1.10 $\pm$ 0.03	30.3 $\pm$ 0.5
100	36.2 $\pm$ 0.3	0.34 $\pm$ 0.01	1.10 $\pm$ 0.04	29.9 $\pm$ 0.7
150	36.9 $\pm$ 0.3	0.33 $\pm$ 0.01	1.15 $\pm$ 0.05	31.1 $\pm$ 0.7
250	35.5 $\pm$ 0.3	0.34 $\pm$ 0.02	1.04 $\pm$ 0.03	31.2 $\pm$ 0.4
400	37.1 $\pm$ 0.4	0.39 $\pm$ 0.03	1.23 $\pm$ 0.05	30.5 $\pm$ 1.2

## **5. VARYING LONG-TERM EXPOSURE DURATIONS**

### **5.1 Introduction**

Having found that long-term exposures at concentrations as low as 50 ppb could cause significant effects in barley and field pea, it was decided to determine the critical duration of exposure that would cause significant effects. No effects were found in plants exposed to ethylene for up to 12 hours (Section 3.0). Therefore, the shortest exposures were set at 3 days for these experiments. In these experiments, concentration was maintained constant and duration was varied.

### **5.2 Exposure Regimes**

Plants were prepared for experimentation as described above. Barley plants were exposed to ethylene at a concentration of 50 ppb for 0, 3, 6, 12, 18 and 24 days while field pea plants were exposed for 0, 12, 16, 20, 24 and 28 days. All treatments were centered around the most sensitive stage as determined in previous studies using ethephon (Archambault and Li 1999). These stages were spike emerging for barley and flat pod for field pea. Following exposure, plants were returned to the greenhouse and grown to maturity.

### **5.3 Results and Discussion**

Exposure of barley plants to ethylene at 50 ppb for up to 24 days had no effect on above-ground (Fig. 5.1) and root biomass (Fig. 5.2). Plant height and tiller numbers were also unaffected by treatment with ethylene (Table 5.1). The number of heads was only significantly reduced when plants were exposed to ethylene for 24 days but head weights started decreasing significantly after as few as 6 days of exposure suggesting that head sizes could have diminished or that heads contained few seeds (Table 5.1). The number of seeds decreased by 27% in 3-day exposures and by 86% in 24-day exposures (Table 5.1). Seed yields were decreased by 41% when plants were exposed to ethylene for 3 days and by as much as 89% when plants were exposed to ethylene for 24 days (Fig. 5.3). Reductions in seed yield were also caused by reductions in seed size (Table 5.2). Exposures of up to 12 days had no effect on seed protein; calcium and phosphorus and caloric contents were unaffected in all treatments (Table 5.2).

In this experiment, nicotine treatment was used on all plants after the longest ethylene exposure. While the treatment was at night (a time when the plants are less sensitive to ethylene, see section 6) and after the most sensitive stages, the potential for an effect due to the additional exposure to ethylene during nicotine treatment cannot be completely ruled out. However, multiple exposures to ethylene at a concentration of 200 ppb at night (section 6) had no effect on plants.

Similarly to barley, exposure of field pea plants to ethylene at 50 ppb for up to 24 days had no effect on above-ground (Fig. 5.4) and root biomass (Fig. 5.5). No significant effects of ethylene on plant height and stem thickness were observed (Table 5.3). Number of pods, weight of pods, number of seeds (Table 5.3), seed yields (Fig. 5.6) and size (Table 5.4) were also unaffected. Long-term exposures to ethylene did not affect seed protein, phosphorus and caloric contents (Table 5.4). Calcium content of seeds was increased by all ethylene treatments (Table 5.4).

These experiments showed that while field pea was relatively insensitive to long-term exposures to ethylene at 50 ppb, barley yields could be reduced significantly in as few as 3 days of exposure at 50 ppb. Reid and Watson (1985) found that in oat, floret numbers were reduced by 84 and 99% when plants were treated with ethylene for 100 days at 70 and 150 ppb, respectively.

#### 5.4 Conclusions

The response of plants to ethylene exposure depends on both concentration and length of exposure. Minimum ethylene concentrations and length of exposure required to cause an effect is species-specific. While field pea did not respond to long-term exposures to ethylene at 50 ppb, barley yields were reduced significantly when exposed to 50 ppb ethylene for 3 days.

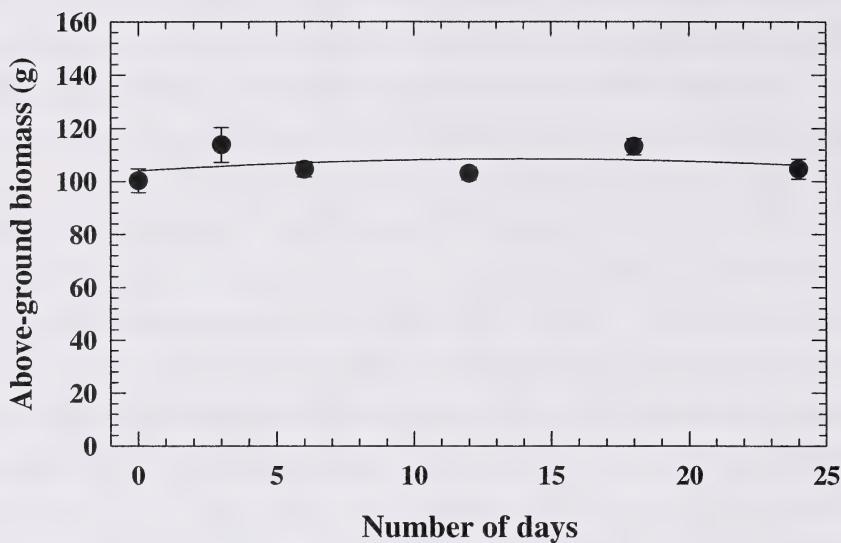


Figure 5.1. Effects of exposure to ethylene at 50 ppb for various lengths of time on above-ground biomass in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. N = 6.

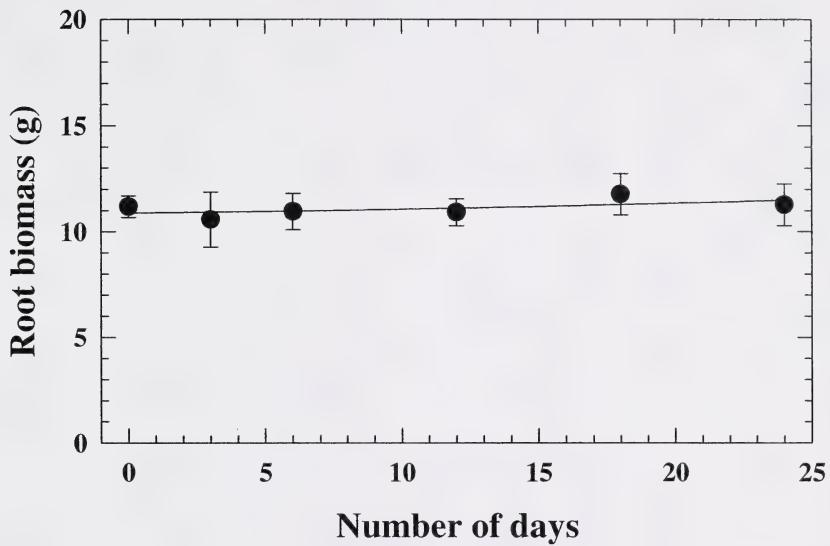


Figure 5.2. Effects of exposure to ethylene at 50 ppb for various lengths of time on root biomass in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. N = 6.

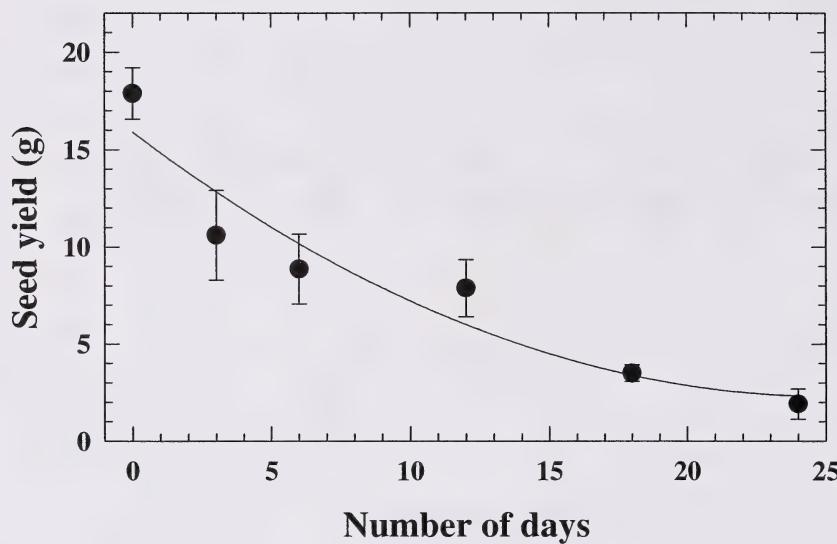


Figure 5.3. Effects of exposure to ethylene at 50 ppb for various lengths of time on seed yield in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. N = 6.

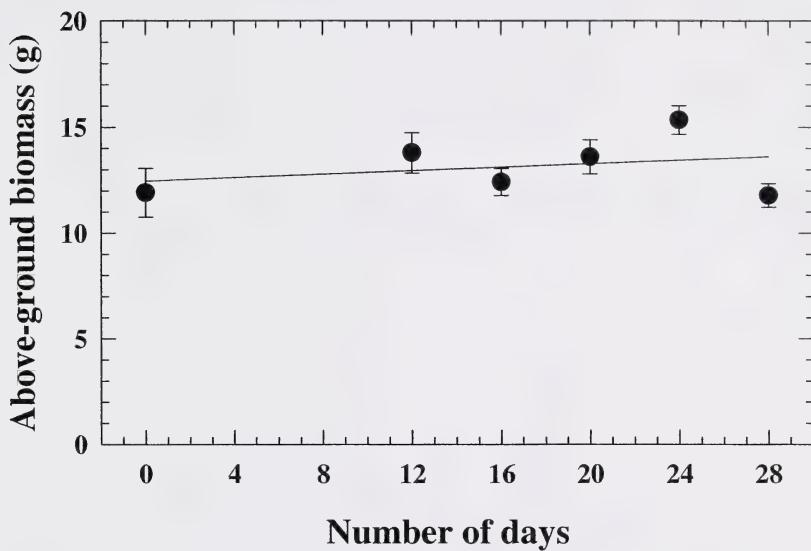


Figure 5.4. Effects of exposure to ethylene at 50 ppb for various lengths of time on above-ground biomass in field pea cv. Carrera. Plants were exposed to ethylene starting at the first buds visible stage. Values are means  $\pm$  S.E. N = 6.

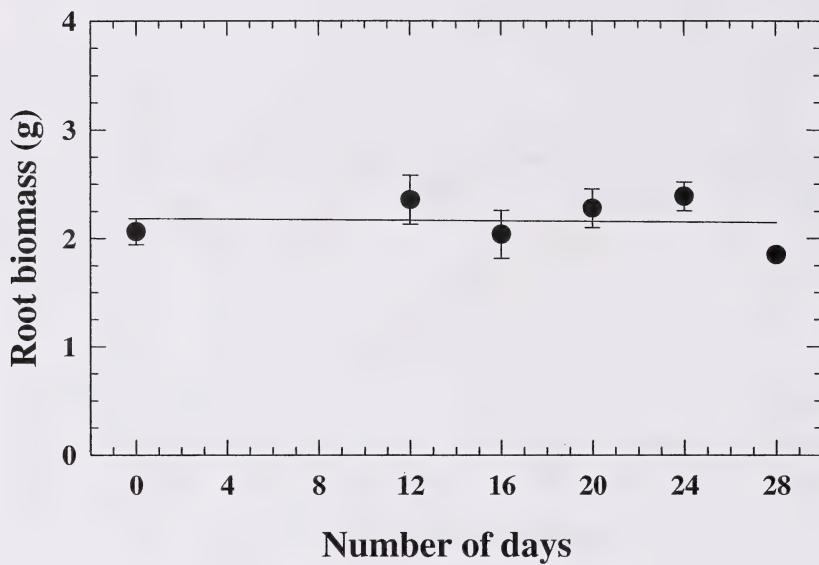


Figure 5.5. Effects of exposure to ethylene at 50 ppb for various lengths of time on root biomass in field pea cv. Carrera. Plants were exposed to ethylene starting at the first buds visible stage. Values are means  $\pm$  S.E. N = 6.

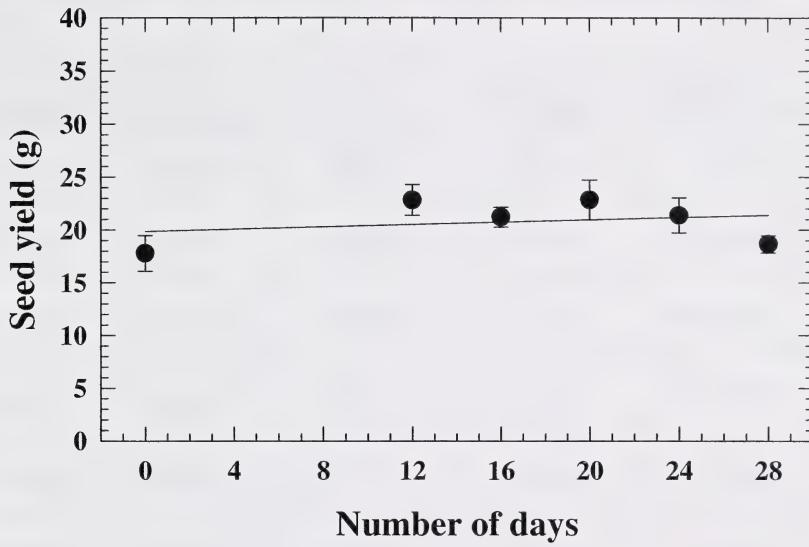


Figure 5.6. Effects of exposure to ethylene at 50 ppb for various lengths of time on seed yield in field pea cv. Carrera. Plants were exposed to ethylene starting at the first buds visible stage. Values are means  $\pm$  S.E. N = 6.

Table 5.1. Effects of exposure to ethylene at 50 ppb for various lengths of time on vegetative and reproductive characters in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Plant heights, N = 18; all others, N = 6.

Treatment	Plant height (cm)	Number of tillers	Number of heads	Weight of heads (g)	Number of seeds
control	78.9 $\pm$ 0.8	201 $\pm$ 16	63 $\pm$ 6	28.5 $\pm$ 1.8	486 $\pm$ 40
3 days	78.9 $\pm$ 0.8	163 $\pm$ 19	78 $\pm$ 5	23.0 $\pm$ 3.4	353 $\pm$ 52
6 days	78.0 $\pm$ 0.9	182 $\pm$ 14	65 $\pm$ 9	18.4 $\pm$ 3.1	312 $\pm$ 67
12 days	78.4 $\pm$ 1.0	188 $\pm$ 6	75 $\pm$ 6	17.6 $\pm$ 2.7	283 $\pm$ 62
18 days	75.6 $\pm$ 1.1	211 $\pm$ 8	96 $\pm$ 24	12.0 $\pm$ 2.2	125 $\pm$ 18
26 days	75.6 $\pm$ 1.1	209 $\pm$ 11	46 $\pm$ 5	8.0 $\pm$ 1.5	68 $\pm$ 23

Table 5.2. Effects of exposure to ethylene at 50 ppb for various lengths of time on seed quality in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. N = 3 to 6. I.S. = insufficient sample for analysis.

Treatment	Weight per 1000 seeds (g)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	Calories per gram DW
control	36.9 $\pm$ 0.8	24.7 $\pm$ 0.8	0.13 $\pm$ 0.01	0.68 $\pm$ 0.01	4383 $\pm$ 7
3 days	29.4 $\pm$ 3.7	22.5 $\pm$ 0.1	0.12 $\pm$ 0.00	0.67 $\pm$ 0.01	4398 $\pm$ 20
6 days	28.6 $\pm$ 2.4	27.9 $\pm$ 2.0	0.15 $\pm$ 0.02	0.77 $\pm$ 0.05	4408 $\pm$ 13
12 days	29.7 $\pm$ 2.2	27.0 $\pm$ 2.5	0.14 $\pm$ 0.01	0.74 $\pm$ 0.04	4388 $\pm$ 18
18 days	28.6 $\pm$ 2.1	I.S.	I.S.	I.S.	4390 $\pm$ 16
26 days	25.6 $\pm$ 2.0	I.S.	I.S.	I.S.	4362 $\pm$ 11

Table 5.3. Effects of exposure to ethylene at 50 ppb for various lengths of time on vegetative and reproductive characters in field pea cv. Carrera. Ethylene exposures started at the first buds visible stage. Values are means  $\pm$  S.E. N = 6.

Treatment	Plant height (cm)	Stem thickness (mm)	Number of pods	Weight of pods (g)	Number of seeds
control	57.4 $\pm$ 1.5	1.46 $\pm$ 0.04	24 $\pm$ 3	21.2 $\pm$ 2.0	90 $\pm$ 9
12 day	56.1 $\pm$ 1.9	1.24 $\pm$ 0.11	27 $\pm$ 2	26.5 $\pm$ 1.7	97 $\pm$ 6
16 days	52.7 $\pm$ 1.4	1.43 $\pm$ 0.16	24 $\pm$ 2	24.9 $\pm$ 1.1	87 $\pm$ 4
20 days	57.7 $\pm$ 2.8	1.54 $\pm$ 0.10	27 $\pm$ 2	27.0 $\pm$ 2.1	96 $\pm$ 6
24 days	59.6 $\pm$ 1.7	1.42 $\pm$ 0.11	32 $\pm$ 3	25.7 $\pm$ 1.8	97 $\pm$ 6
28 days	54.1 $\pm$ 3.0	1.49 $\pm$ 0.17	23 $\pm$ 1	22.0 $\pm$ 0.8	81 $\pm$ 4

Table 5.4. Effects of exposure to ethylene at 50 ppb for various lengths of time on seed quality in field pea cv. Carrera. Ethylene exposures started at the first buds visible stage. Values are means  $\pm$  S.E. N = 6.

Treatment	Weight per 1000 seeds (g)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	Calories per gram DW
<b>control</b>	199.6 $\pm$ 9.4	26.0 $\pm$ 0.4	0.11 $\pm$ 0.01	0.59 $\pm$ 0.01	4209 $\pm$ 6
<b>12 day</b>	236.1 $\pm$ 3.8	26.6 $\pm$ 0.4	0.15 $\pm$ 0.01	0.61 $\pm$ 0.01	4222 $\pm$ 10
<b>16 days</b>	244.0 $\pm$ 8.2	27.6 $\pm$ 0.4	0.15 $\pm$ 0.02	0.64 $\pm$ 0.01	4239 $\pm$ 9
<b>20 days</b>	237.5 $\pm$ 9.1	26.7 $\pm$ 0.5	0.18 $\pm$ 0.01	0.61 $\pm$ 0.01	4218 $\pm$ 9
<b>24 days</b>	221.2 $\pm$ 9.6	26.1 $\pm$ 0.5	0.14 $\pm$ 0.0	0.60 $\pm$ 0.00	4212 $\pm$ 7
<b>28 days</b>	232.3 $\pm$ 7.1	26.5 $\pm$ 0.4	0.16 $\pm$ 0.01	0.63 $\pm$ 0.01	4216 $\pm$ 14

## **6. TIME OF DAY EXPOSURES**

### **6.1 Introduction**

Thus far, experiments had been designed on the assumption that plants were evenly sensitive to ethylene throughout the day and night. Tonneijck et al. (1999, 2000) observed that ethylene-induced epinastic responses of potato plants grown near polyethylene manufacturing plants were greater at night and concluded that plants were most sensitive to ethylene at that time of day. Therefore, the following experiment was designed to test whether sensitivity of barley to ethylene varied with time of day.

### **6.2 Exposure Regimes**

Barley plants were prepared for experimentation as described above. Each 24-hour period was segmented into 4 six-hour periods and exposures to ethylene were repeated daily for 30 days.

Plants were either kept at control conditions (10 ppb ethylene) or exposed daily to ethylene at a concentration of 200 ppb for 6-hour periods from either 4 a.m. to 10 a.m. (spanning dark to light transition), 10a.m. to 4 p.m., 4 p.m. to 10 p.m. (both full light) or 10 p.m. to 4 a.m. (full dark).

Plants were kept at control conditions (10 ppb) for periods between exposures at 200 ppb.

Following exposure, plants were returned to the greenhouse and grown to maturity.

### **6.3 Results and Discussion**

Intermittent exposures of barley to ethylene at 200 ppb for 6 hours per day generally caused increases in above-ground (Fig. 6.1) and root (Fig. 6.2) biomass. Plant heights and number of tillers were unaffected (Table 6.1). Number of heads, weight of heads and number of seeds were reduced when plants were exposed to ethylene between 10:00 a.m. and 4:00 p.m. (Table 6.1). A reduction of 50% in seed yield was also observed (Fig. 6.3). In experiments described in section 4, 14 days of continuous exposure to ethylene at 100 ppb and above caused near complete loss of yield (Fig. 4.7). The discrepancy between the findings reported in section 4 and those reported here suggest that while plants responded differentially to exposures at different times of day, the intermittent nature of the exposures allowed for recovery in seed yield. This reduction appears to be solely attributable to seed numbers as no effects on seed size were observed (Fig. 6.2). This observation is also in contrast with those from previous experiments that showed that reductions in yield were partially attributable to reductions in seed size (Tables 4.2 and 5.2). It appears possible that in intermittent exposure scenarios, recovery of seed

size can be complete but not in seed number. No effects on protein, calcium and phosphorus content of seeds were observed (Table 6.2). Caloric content was only significantly decreased in plants exposed to ethylene between 10:00 p.m. and 4:00 a.m. Greater effect on plants exposed to ethylene from 10:00 a.m. and 4:00 p.m. coincides with reported increases in endogenous production of ethylene in seedlings of barley (Kurapov et al. 2000) and sorghum (Finlayson et al. 1998, 1999) during that time of day and with natural levels of ethylene in air in certain pristine environments (Reid and Watson 1985). In contrast, Tonneijck et al. (2000) found greater epinastic responses of potato leaves exposed to ethylene at nighttime. Differences in sensitivity to time of day of exposure may be because of species differences; it may also be associated with the fact that different parameters were observed in these two studies.

In this experiment, plants were treated with nicotine 10, 17 and 35 days prior to the first exposure to ethylene. All of these treatments were applied before the onset of the most ethylene-sensitive stages. The nicotine treatments would have added 3 nighttime exposures approximately equivalent to the dose of 1.5 hours and 120 ppb. The present experiment showed that 30 nighttime exposures of ethylene at 200 ppb had no effect on plants. Therefore, it is unlikely that the nicotine treatments caused any effects that would affect the interpretation of the results.

#### **6.4 Conclusions**

Time of day of exposure is an important factor in determining the relative sensitivities of plants to ethylene. Barley cv. Harrington is most sensitive to ethylene between 10:00 a.m. and 4:00 p.m. Differences between the observations from this study and those from the literature suggest that diurnal sensitivity patterns are species-specific. This further complicates the interpretation of the effects of continuous and intermittent exposures.

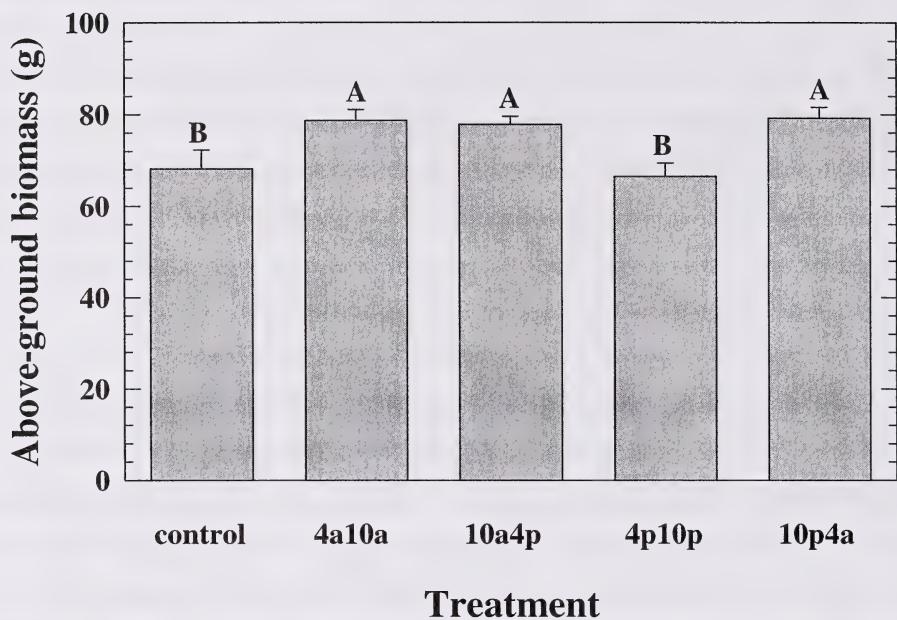


Figure 6.1. Effects of exposure to ethylene at 200 ppb at various times of day on above-ground biomass in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Bars with no letters in common are significantly different at  $P \geq 0.05$ .  $N = 6$ .

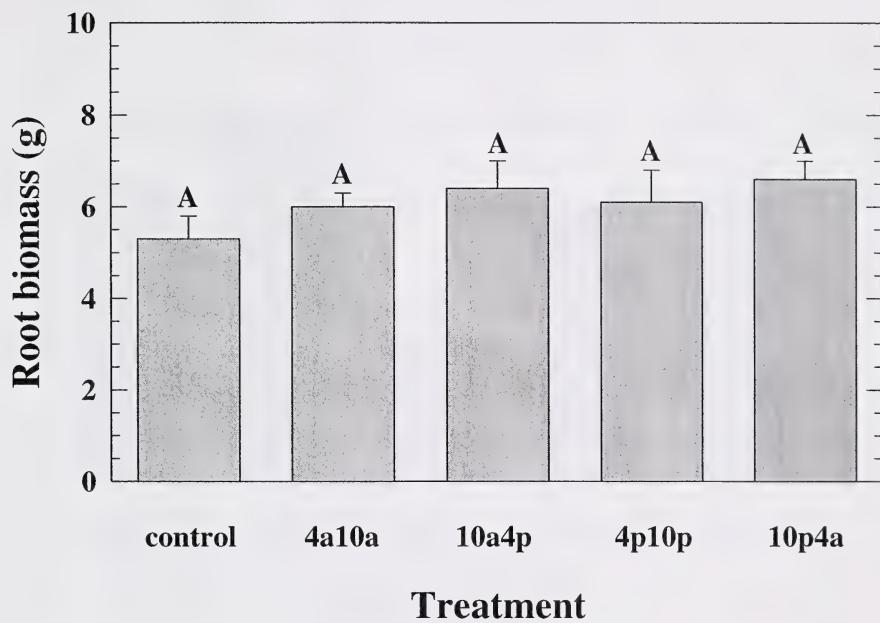


Figure 6.2. Effects of exposure to ethylene at 200 ppb at various times of day on root biomass in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Bars with no letters in common are significantly different at  $P \geq 0.05$ .  
N = 6.

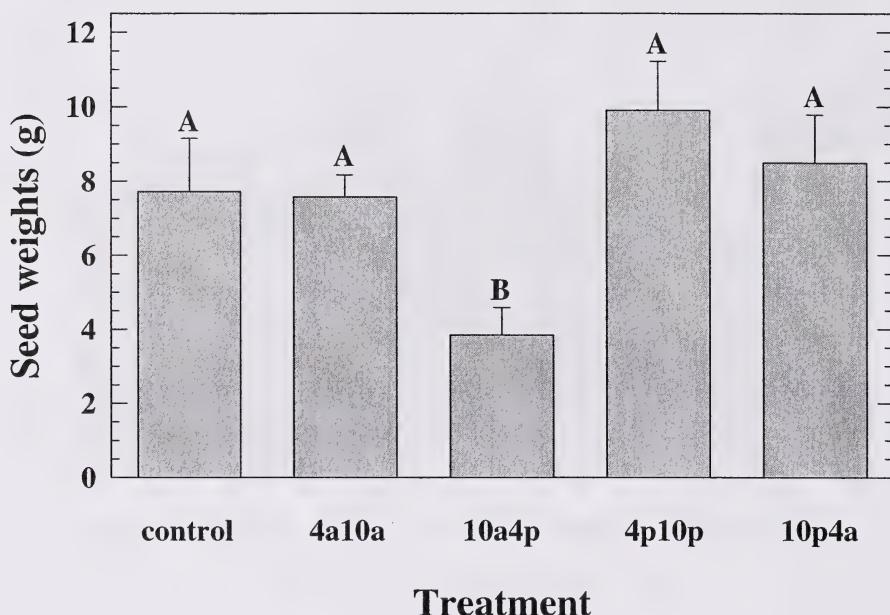


Figure 6.3. Effects of exposure to ethylene at 200 ppb at various times of day on seed yield in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Bars with no letters in common are significantly different at  $P \geq 0.05$ .  
N = 6.

Table 6.1. Effects of exposure to ethylene at 200 ppb at various times of day on vegetative and reproductive characters in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . Plant heights,  $N = 18$ ; all others,  $N = 6$ .

Treatment	Plant height (cm)	Number of tillers	Number of heads	Weight of heads (g)	Number of seeds
control	$67.9 \pm 0.8$	$150 \pm 10$	$68 \pm 10$	$15.6 \pm 2.6$	$259 \pm 49$
	<b>AB</b>	<b>A</b>	<b>AB</b>	<b>A</b>	<b>A</b>
4a.m. -	$67.7 \pm 0.7$	$172 \pm 7$	$75 \pm 8$	$16.4 \pm 1.3$	$230 \pm 17$
10a.m.	<b>AB</b>	<b>A</b>	<b>AB</b>	<b>A</b>	<b>A</b>
10a.m. -	$65.2 \pm 0.9$	$158 \pm 11$	$49 \pm 9$	$8.7 \pm 1.5$	$125 \pm 23$
4p.m.	<b>BC</b>	<b>A</b>	<b>B</b>	<b>B</b>	<b>B</b>
4p.m. -	$63.6 \pm 1.0$	$161 \pm 13$	$82 \pm 9$	$16.9 \pm 1.8$	$327 \pm 37$
10p.m.	<b>C</b>	<b>A</b>	<b>A</b>	<b>A</b>	<b>A</b>
10p.m. -	$68.1 \pm 0.9$	$163 \pm 6$	$67 \pm 5$	$15.7 \pm 1.8$	$257 \pm 37$
4a.m.	<b>AB</b>	<b>A</b>	<b>AB</b>	<b>A</b>	<b>A</b>

Table 6.2. Effects of exposure to ethylene at 200 ppb at various times of day on seed quality in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 6.

Treatment	Weight per 1000 seeds (g)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	Calories per gram DW
control	28.3 $\pm$ 0.7 <b>A</b>	34.1 $\pm$ 0.8 <b>A</b>	0.17 $\pm$ 0.01 <b>A</b>	0.68 $\pm$ 0.01 <b>A</b>	4165 $\pm$ 11 <b>A</b>
4a.m. -	29.8 $\pm$ 0.6 <b>A</b>	32.1 $\pm$ 0.9 <b>A</b>	0.16 $\pm$ 0.01 <b>A</b>	0.68 $\pm$ 0.02 <b>A</b>	4144 $\pm$ 10 <b>AB</b>
10a.m. -	32.9 $\pm$ 0.4 <b>A</b>	31.3 $\pm$ 0.6 <b>A</b>	0.16 $\pm$ 0.01 <b>A</b>	0.66 $\pm$ 0.01 <b>A</b>	4155 $\pm$ 8 <b>AB</b>
10p.m. -	30.2 $\pm$ 1.0 <b>A</b>	33.7 $\pm$ 0.9 <b>A</b>	0.20 $\pm$ 0.02 <b>A</b>	0.69 $\pm$ 0.02 <b>A</b>	4141 $\pm$ 6 <b>AB</b>
4p.m. -	29.8 $\pm$ 1.2 <b>A</b>	30.8 $\pm$ 1.2 <b>A</b>	0.17 $\pm$ 0.01 <b>A</b>	0.65 $\pm$ 0.02 <b>A</b>	4113 $\pm$ 8 <b>C</b>

## **7. RESPONSES OF BARLEY TO A DEMONSTRATION PATTERN DEVELOPED FROM AIR QUALITY MONITORING DATA**

### **7.1 Introduction**

In this experiment, a 30-day exposure based on monitoring data submitted by industry to Alberta Environment was performed using barley. The exposure regime was developed by selecting the month in which the highest average ethylene concentration was reported (September, 1995; Joffre, Trailer 351). For each period longer than 1-hour in which ethylene concentrations were above 5 ppb: the period was set, and the concentration for that period was assigned as being the highest reported 1-hour average within that period, rounded to the nearest 10 ppb. Any monitoring value below 5 ppb was assigned a background concentration of 10 ppb. Based upon these criteria, the profile shown in Figure 7.1 was generated. This approach gave a demonstration pattern of exposure for plants grown near a fenceline of an ethylene facility.

### **7.2 Exposure Regimes**

Barley plants were prepared for experimentation as described above. Plants were either kept at control conditions (10 ppb ethylene) or exposed to a demonstration pattern (Figure 7.1). Following exposure, plants were returned to the greenhouse and grown to maturity.

### **7.3 Results and Discussion**

Barley plants exposed to the demonstration pattern had greater above-ground (Fig. 7.2) and root biomass (Table 7.1) than control plants. No effects on plant height, number of tillers, number of heads, weight of heads, number of seeds (Table 7.1), seed yield (Figure 7.1), seed size (Table 7.2) and seed quality (Table 7.2) were observed. Significant increases in above- and below-ground biomass suggest that plants did perceive and respond to ethylene, however, the ethylene exposure was not great enough to have detrimental effects on reproductive characters. Intermittent exposures to ethylene may cause increases in biomass while not affecting seed yields. Increases in biomass may be beneficial if barley is to be harvested for silage.

This experiment was conducted concurrently with that described in section 6 and plants were treated with nicotine 3 times. No significant effects of multiple exposures to ethylene on seed

yield were observed in this experiment. Therefore, it is unlikely that the nicotine treatments caused any effects that would affect the interpretation of the results.

#### **7.4 Conclusions**

Exposure of barley plants to the demonstration pattern at the most sensitive stages of development had no significant effect on reproductive parameters but caused an increase in above- and below-ground biomass.

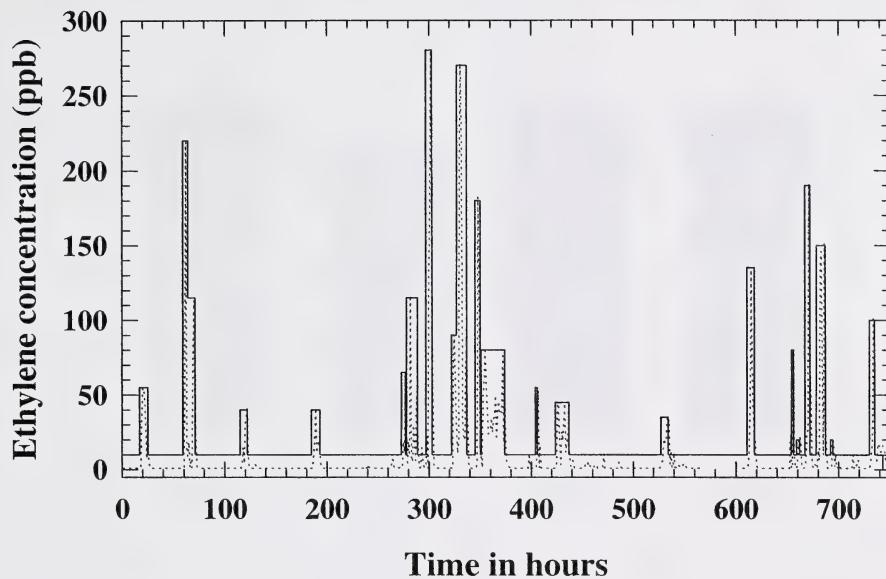


Figure 7.1. Demonstration pattern of ethylene exposure (solid line) and monitoring data used to produce the pattern (dashed line). A description of the significance of this pattern is given in section 7.1.

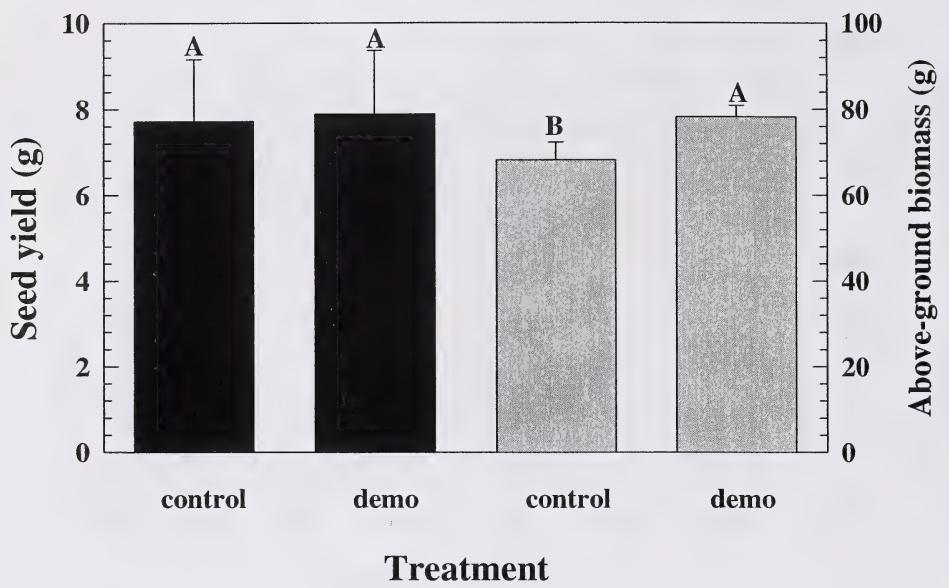


Figure 7.2. Effects of exposure to ethylene in a 30-day demonstration pattern on seed yield and above-ground biomass in barley cv. Harrington. Ethylene exposure was centered around the spike emerging stage. Columns of the same colour bearing no letters in common are significantly different at  $P \geq 0.05$ . Values are means  $\pm$  S.E. N = 6.

Table 7.1. Effects of exposure to ethylene in a demonstration pattern on vegetative and reproductive characters in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . Plant heights, N = 18; all others, N = 6.

Treatment	Plant height (cm)	Number of tillers	Root biomass (g)	Number of heads	Weight of heads (g)	Number of seeds
control	67.9 $\pm$ 0.8 A	150 $\pm$ 10 A	5.34 $\pm$ 0.53 B	68 $\pm$ 10 A	15.6 $\pm$ 2.6 A	259 $\pm$ 49 A
Demo pattern	69.7 $\pm$ 2.3 A	167 $\pm$ 9 A	7.44 $\pm$ 0.45 A	70 $\pm$ 11 A	15.3 $\pm$ 2.3 A	254 $\pm$ 39 A

Table 7.2. Effects of exposure to ethylene in a demonstration pattern on seed quality in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 6.

Treatment	Weight per 1000 seeds (g)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	Calories per gram DW
control	28.3 $\pm$ 0.7 A	34.1 $\pm$ 0.8 A	0.17 $\pm$ 0.01 A	0.68 $\pm$ 0.01 A	4165 $\pm$ 11 A
Demo pattern	29.4 $\pm$ 2.4 A	32.3 $\pm$ 1.8 A	0.19 $\pm$ 0.02 A	0.70 $\pm$ 0.05 A	4136 $\pm$ 8 A

## **8. EXPOSURE INTERVAL LENGTH AND PLANT RECOVERY**

### **8.1 Introduction**

The experiment described in section 6 showed that multiple intermittent exposures to ethylene at 200 ppb from 10:00 a.m. to 4:00 p.m. caused significant decreases in seed yields in barley. Exposures in that experiment were repeated daily with no interval longer than 18 hours between exposures. The results in section 7 also show that intermittent exposures cause plant responses. It therefore remained to determine the potential importance of interval length on the observed effects. In the experiments described below, an attempt was made at establishing the critical interval length that would allow for full plant recovery.

### **8.2 Exposure Regimes**

Barley plants were prepared for experimentation as described above. Plants were either kept at control conditions (10 ppb ethylene continuous) or exposed to ethylene at a concentration of either 250 or 500 ppb for 6-hour periods from 10:00 a.m. to 4:00 p.m. repeated five times at intervals of 0, 1, 3, and 5 days. In the case of the 11-day interval, the exposures were repeated three times. Plants were kept at control conditions (10 ppb) for periods between exposures at 250 or 500 ppb. Following exposure, plants were returned to the greenhouse and grown until harvest.

### **8.3 Results and Discussion**

The results presented here are from three experiments all of which, while somewhat different in design, aimed at understanding the effects of the length of intervals between ethylene exposures on plant response. It was necessary to use multiple trials to this end because of a number of technical difficulties that arose in the conduct of the experiments. In the first trial, growth chamber failure caused lights to remain on 24 hours per day in the control and 1-day interval treatments. While usable controls were not available in this trial, comparisons could be made between some of the treatments on a relative basis. In the second trial, insect attack led to questionable results for two treatments. In the last trial, a fungal outbreak caused the loss of a duplicate set of controls that were inserted into the experimental design to attempt to ensure success of the trial. Despite these difficulties, useful and interesting conclusions can be drawn from this set of experiments. Standardized data (standardized to the 0-day interval treatment

that was common to all experiments) were pooled to attempt to clarify the effects of interval length on plant response.

In the first experiment, plant heights were significantly reduced in all treatments relative to controls (Table 8.1) perhaps due to reduced internode elongation (Table 8.1). No significant effects on above-ground biomass, root biomass and tiller numbers were observed (Table 8.1). Seed yields of plants grown at control conditions and those exposed to ethylene at 1-day intervals were significantly greater than seed yields of plants exposed to ethylene at 0-, 3-, 5- and 11-day intervals (Fig. 8.1). This appears to be predominantly due to increased seed numbers and size rather than number of heads (Table 8.2). Weights of heads were significantly lower in plants exposed to ethylene at 0- and 3-day intervals relative to controls (Table 8.2). While as described above, controls and 1-day interval data were compromised in this experiment, we can conclude that intervals as long as 11 days do not allow for significant recovery relative to shorter interval lengths. No effects on seed quality were observed (Table 8.3).

In the second experiment, plant heights were decreased in all treatments relative to controls (Table 8.4). Above-ground biomass was only significant in plants exposed to 250 ppb ethylene at 3-day intervals but no significant effects on root biomass were observed in any of the treatments (Table 8.4). Tiller numbers were increased in all ethylene treatments but were only statistically significant for plants exposed to 250 ppb ethylene at 0-day intervals and to 500 ppb ethylene at 1-day intervals (Table 8.4). Number of heads, weight of heads and number of seeds were generally decreased in plants exposed to ethylene (Table 8.4). In all cases, the lowest values were obtained in plants exposed to 500 ppb ethylene at 3-day intervals but, as described above, this appeared to be caused by insect attack rather than ethylene treatment. Seed yields were decreased in all ethylene treatments (Fig. 8.2). Those of plants treated with ethylene at 250 ppb at 0-, 1- and 3-day intervals were significantly lower than controls and a general trend towards decreased effect of ethylene with increased interval length was observed (Fig. 8.2). Seed size was only significantly decreased in plants treated with 500 ppb ethylene at 3-day intervals (Table 8.5) again possibly exhibiting symptoms of insect attack. No significant effects on seed protein, calcium and phosphorus were observed (Table 8.5). Caloric content of seeds

was significantly reduced in plants treated with 500 ppb ethylene at 1-day intervals relative to controls (Table 8.5). Other samples were not analyzed to avoid redundancy with the previous experiment.

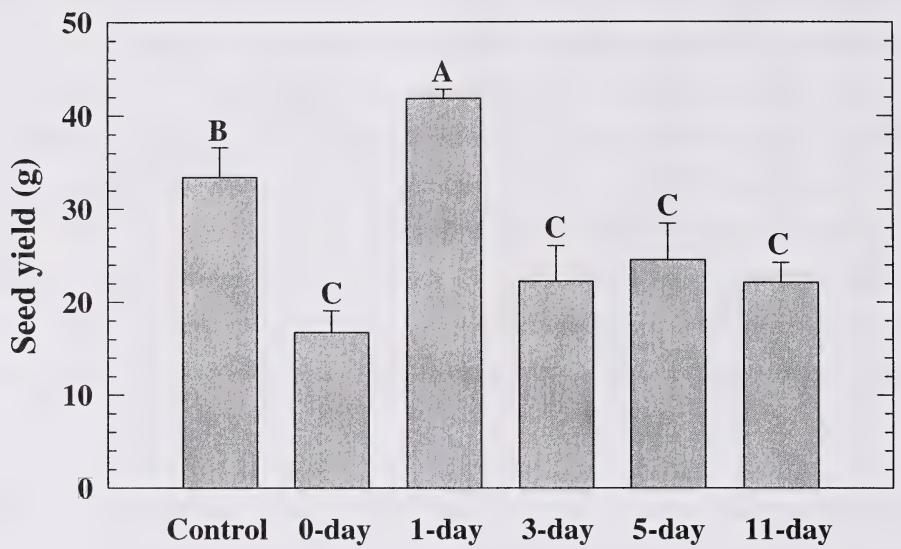
In the last experiment, plant heights were significantly reduced in plants exposed to ethylene at 1-, 3- and 5-day intervals possibly due to reductions in internode length (Table 8.6). While no effects on root biomass and number of tillers were observed, above-ground biomass was reduced in plants exposed to ethylene at 5-day intervals (Table 8.6). Seed yields were significantly reduced in plants exposed to ethylene at 0-, 1- and 5-day intervals (Fig. 8.3). This appears to be independent of the number of heads formed and size of heads but dependent on seed number and size (Table 8.7). No effects of ethylene on seed quality were observed (Table 8.8).

All usable (non-compromised) seed yield data were standardized to the 0-day data and pooled in order to ease interpretation of the results of these three trials (Fig. 8.4). Taken together, the data suggest that exposure of plants to five intermittent 6-hour episodes of ethylene at 250 ppb caused a reduction in yield and intervals as long as 5 days were not sufficient to allow for complete recovery. The total ethylene dose received by plants in these experiments is comparable to those of the long-term experiments described in sections 4 and 5, yet yield reductions were less. This suggests that the dose equation (concentration x duration) may not apply to intermittent exposures and that some recovery occurs between successive exposures. Tonneijck et al. (2000) and Van Raay (1980) also reported that intermittent exposures to ethylene were less effective in causing epinastic responses and reducing tuber yield of potato, respectively, than continuous exposures.

In this set of experiments, plants from the second and third trials were treated with nicotine at least 10 days prior to the first exposure to ethylene. All of these treatments were applied before the onset of the most ethylene-sensitive stages. The experiments in section 6 showed that 30 nighttime exposures of ethylene at 200 ppb had no effect on plants. Therefore, it is unlikely that the nicotine treatments caused any effects that would affect the interpretation of the results.

#### **8.4 Conclusions**

Exposure of barley plants to 5 intermittent exposures of ethylene at 250 ppb caused a reduction in yield and intervals as long as 5 days are not sufficient to allow for complete recovery. The dose equation (concentration x duration) may not apply to intermittent exposures.



### Length of interval between ethylene treatments

Figure 8.1. Effects of repeated exposures to ethylene at 250 ppb at various interval lengths on seed yield in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Bars with no letters in common are significantly different at  $P \geq 0.05$ .  $N = 6$ .

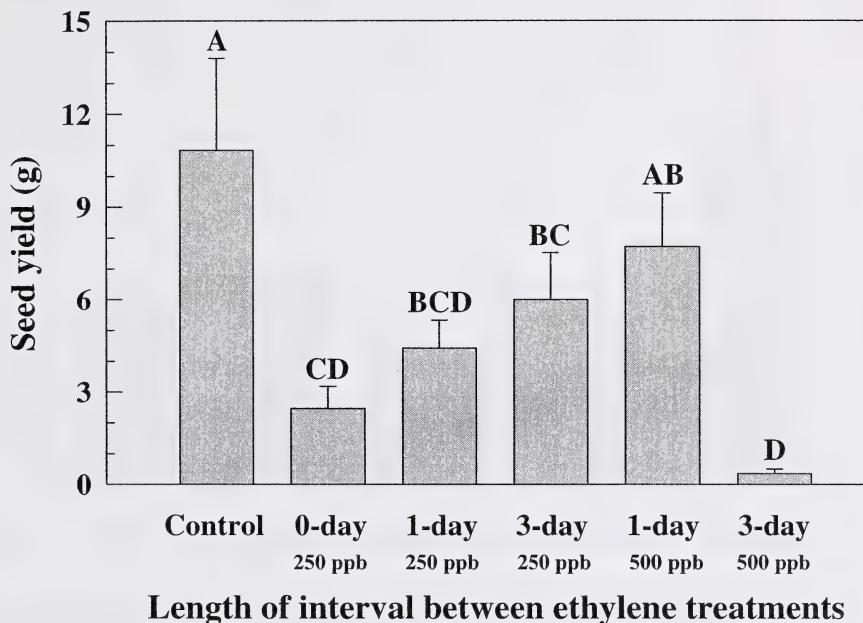


Figure 8.2. Effects of repeated exposures to ethylene at 250 ppb or 500 ppb at various interval lengths on seed yield in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Bars with no letters in common are significantly different at  $P \leq 0.05$ . N = 6.

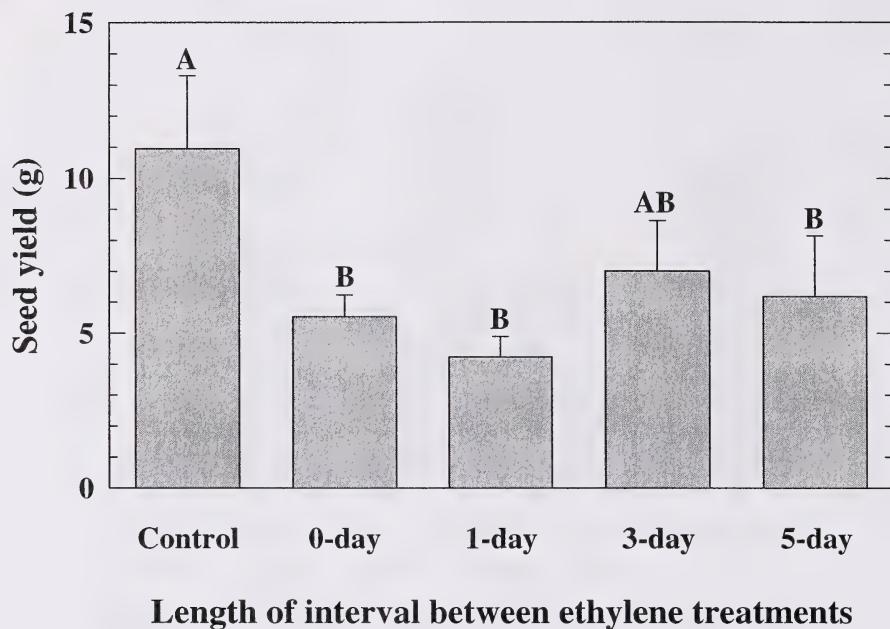


Figure 8.3. Effects of repeated exposures to ethylene at 250 ppb at various interval lengths on seed yield in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Bars with no letters in common are significantly different at  $P \geq 0.05$ .  $N = 6$ .

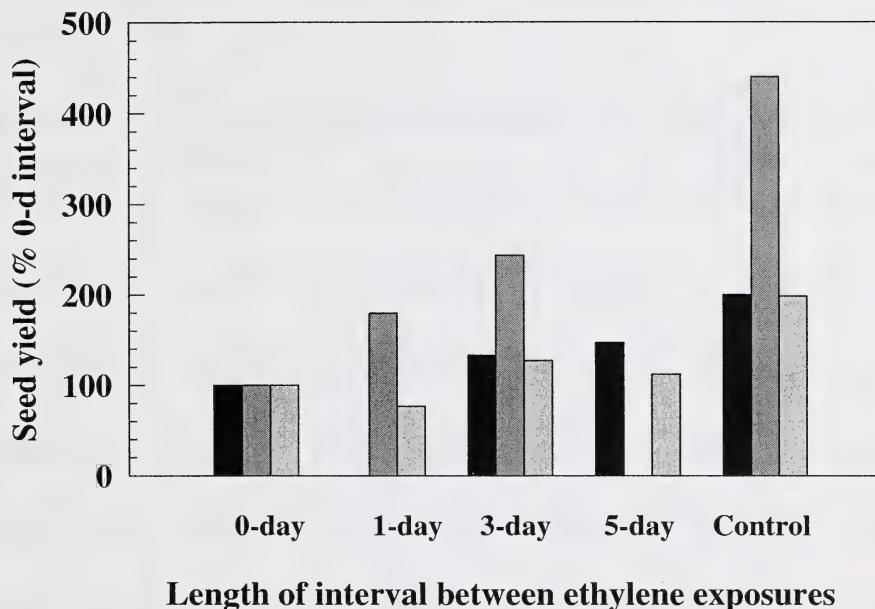


Figure 8.4. Effects of repeated exposures to ethylene at 250 ppb at various interval lengths on seed yield in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Data are pooled from three experiments and standardized to the 0-day interval values. Bars represent the first (dark gray), the second (black), the third (light gray) experiments. N = 6.

Table 8.1. Effects of length of interval between exposures to ethylene at 250 ppb on vegetative characters in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 6.

Length of intervals	Plant height (cm)	AG biomass (g)	Root biomass (g)	Number of tillers	Internode length (cm)
control	92.6 $\pm$ 1.1 A	100.3 $\pm$ 3.9 A	9.4 $\pm$ 0.9 AB	150 $\pm$ 16 A	12.3 $\pm$ 0.3 A
0 days	84.6 $\pm$ 1.1 C	101.3 $\pm$ 5.6 A	9.9 $\pm$ 1.1 AB	172 $\pm$ 23 A	10.4 $\pm$ 0.4 B
1 day	88.5 $\pm$ 0.9 B	87.1 $\pm$ 1.7 A	9.6 $\pm$ 0.6 AB	152 $\pm$ 7 A	11.3 $\pm$ 0.2 AB
3 days	82.5 $\pm$ 1.3 C	95.7 $\pm$ 11.2 A	8.1 $\pm$ 1.2 B	154 $\pm$ 21 A	11.0 $\pm$ 0.3 B
5 days	88.2 $\pm$ 0.9 B	103.9 $\pm$ 3.1 A	11.6 $\pm$ 0.3 A	164 $\pm$ 16 A	11.0 $\pm$ 0.5 B
11 days	82.6 $\pm$ 0.8 C	96.9 $\pm$ 8.6 A	9.4 $\pm$ 1.2 AB	154 $\pm$ 23 A	10.4 $\pm$ 0.6 B

Table 8.2. Effects of length of interval between exposures to ethylene at 250 ppb on reproductive characters in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 6.

Length of intervals	Number of heads	Weight of heads (g)	Number of seeds	Weight per 1000 seeds (g)
control	81 $\pm$ 2 <b>AB</b>	50.6 $\pm$ 3.9 <b>AB</b>	829 $\pm$ 97 <b>AB</b>	41.0 $\pm$ 3.5 <b>A</b>
0 days	76 $\pm$ 4 <b>AB</b>	30.6 $\pm$ 3.5 <b>C</b>	490 $\pm$ 111 <b>C</b>	36.8 $\pm$ 3.0 <b>A</b>
1 day	92 $\pm$ 4 <b>A</b>	58.1 $\pm$ 1.5 <b>A</b>	979 $\pm$ 22 <b>A</b>	42.7 $\pm$ 0.6 <b>A</b>
3 days	81 $\pm$ 11 <b>AB</b>	39.0 $\pm$ 6.4 <b>C</b>	609 $\pm$ 128 <b>BC</b>	34.3 $\pm$ 2.9 <b>A</b>
5 days	70 $\pm$ 4 <b>B</b>	38.9 $\pm$ 5.1 <b>BC</b>	644 $\pm$ 132 <b>BC</b>	39.3 $\pm$ 3.4 <b>A</b>
11 days	83 $\pm$ 6 <b>AB</b>	41.1 $\pm$ 3.4 <b>BC</b>	649 $\pm$ 77 <b>BC</b>	35.0 $\pm$ 2.3 <b>A</b>

Table 8.3. Effects of length of interval between exposures to ethylene at 250 ppb on seed quality in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 6.

Length of intervals	% Protein per DW	% Calcium per DW	% Phosphorus per DW	Calories per gram DW
control	21.4 $\pm$ 0.8 A	0.17 $\pm$ 0.00 A	0.54 $\pm$ 0.01 ABC	4066 $\pm$ 13 A
0 days	21.7 $\pm$ 0.4 A	0.17 $\pm$ 0.00 A	0.57 $\pm$ 0.01 A	4053 $\pm$ 4 A
1 day	20.7 $\pm$ 0.1 A	0.15 $\pm$ 0.00 B	0.50 $\pm$ 0.01 C	4053 $\pm$ 4 A
3 days	21.6 $\pm$ 0.6 A	0.17 $\pm$ 0.00 A	0.51 $\pm$ 0.01 BC	4054 $\pm$ 7 A
5 days	21.7 $\pm$ 0.7 A	0.16 $\pm$ 0.00 A	0.54 $\pm$ 0.02 AB	4047 $\pm$ 9 A
11 days	22.0 $\pm$ 1.0 A	0.17 $\pm$ 0.01 A	0.53 $\pm$ 0.01 ABC	4061 $\pm$ 8 A

Table 8.4. Effects of length of interval between exposures to ethylene at either 250 ppb or 500 ppb on vegetative and reproductive characters in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 6.

Length of intervals	Plant height (cm)	AG biomass (g)	Root biomass (g)	Number of tillers	Number of heads	Weight of heads (g)
<b>control</b>	87.6 $\pm$ 1.0 <b>A</b>	84.8 $\pm$ 4.6 <b>B</b>	7.96 $\pm$ 1.18 <b>A</b>	139 $\pm$ 15 <b>B</b>	52 $\pm$ 7 <b>AB</b>	20.2 $\pm$ 4.4 <b>A</b>
<b>0 days – 250 ppb</b>	74.9 $\pm$ 1.6 <b>C</b>	92.5 $\pm$ 1.8 <b>AB</b>	9.23 $\pm$ 0.79 <b>A</b>	181 $\pm$ 5 <b>A</b>	33 $\pm$ 6 <b>BC</b>	9.3 $\pm$ 2.5 <b>BC</b>
<b>1 day – 250 ppb</b>	77.4 $\pm$ 1.0 <b>C</b>	89.3 $\pm$ 2.8 <b>AB</b>	7.88 $\pm$ 1.17 <b>A</b>	153 $\pm$ 21 <b>AB</b>	42 $\pm$ 9 <b>ABC</b>	10.8 $\pm$ 2.0 <b>BC</b>
<b>3 days – 250 ppb</b>	75.7 $\pm$ 0.8 <b>C</b>	90.4 $\pm$ 1.6 <b>AB</b>	8.25 $\pm$ 0.43 <b>A</b>	167 $\pm$ 2 <b>AB</b>	54 $\pm$ 6 <b>A</b>	14.2 $\pm$ 2.5 <b>AB</b>
<b>1 day – 500 ppb</b>	83.6 $\pm$ 1.0 <b>B</b>	93.8 $\pm$ 2.0 <b>A</b>	8.82 $\pm$ 0.72 <b>A</b>	178 $\pm$ 9 <b>A</b>	49 $\pm$ 6 <b>AB</b>	16.1 $\pm$ 3.2 <b>AB</b>
<b>3 days – 500 ppb</b>	70.6 $\pm$ 1.0 <b>D</b>	90.8 $\pm$ 1.5 <b>AB</b>	10.31 $\pm$ 0.22 <b>A</b>	173 $\pm$ 3 <b>B</b>	24 $\pm$ 4 <b>C</b>	3.1 $\pm$ 0.8 <b>C</b>

Table 8.5. Effects of length of interval between exposures to ethylene at either 250 ppb or 500 ppb on seed numbers and quality in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 3 to 6. I.S. = insufficient sample for analysis. N.A. = no analysis.

Length of intervals	Number of seeds	Weight per 1000 seeds (g)	% Protein per DW	% Calcium per DW	% Phosphorus per DW	Calories per gram DW
control	306 $\pm$ 65 A	32.8 $\pm$ 2.9 A	25.0 $\pm$ 2.1 A	0.17 $\pm$ 0.01 A	0.52 $\pm$ 0.01 AB	4450 $\pm$ 11 A
0 days - 250 ppb	78 $\pm$ 24 CD	32.2 $\pm$ 1.8 A	22.0 $\pm$ 0.4 A	0.16 $\pm$ 0.01 A	0.49 $\pm$ 0.01 B	N.A.
1 day - 250 ppb	133 $\pm$ 19 BCD	32.2 $\pm$ 2.0 A	23.9 $\pm$ 1.6 A	0.17 $\pm$ 0.01 A	0.51 $\pm$ 0.03 AB	N.A.
3 days - 250 ppb	173 $\pm$ 37 BC	33.6 $\pm$ 1.4 A	23.3 $\pm$ 0.4 A	0.16 $\pm$ 0.00 A	0.50 $\pm$ 0.01 B	N.A.
1 day - 500 ppb	225 $\pm$ 45 AB	33.8 $\pm$ 1.2 A	21.0 $\pm$ 0.9 A	0.16 $\pm$ 0.00 A	0.55 $\pm$ 0.01 A	4409 $\pm$ 9 B
3 days - 500 ppb	18 $\pm$ 9 D	16.6 $\pm$ 4.1 B	I.S.	I.S.	I.S.	I.S.

Table 8.6. Effects of length of interval between exposures to ethylene at 250 ppb on vegetative characters in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 6.

Length of intervals	Plant height (cm)	AG biomass (g)	Root biomass (g)	Number of tillers	Internode length (cm)
control	75.7 $\pm$ 0.9 A	80.0 $\pm$ 2.5 A	7.4 $\pm$ 0.7 A	89 $\pm$ 2 A	10.4 $\pm$ 0.4 A
0 days	72.8 $\pm$ 0.8 AB	80.8 $\pm$ 2.4 A	6.4 $\pm$ 0.8 A	90 $\pm$ 6 A	9.1 $\pm$ 0.2 B
1 day	72.1 $\pm$ 1.0 B	78.7 $\pm$ 1.7 AB	6.7 $\pm$ 0.4 A	99 $\pm$ 3 A	9.7 $\pm$ 0.3 AB
3 days	71.6 $\pm$ 1.2 B	80.6 $\pm$ 1.5 A	7.8 $\pm$ 0.5 A	100 $\pm$ 3 A	9.2 $\pm$ 0.3 B
5 days	70.8 $\pm$ 1.3 B	72.3 $\pm$ 3.9 B	5.9 $\pm$ 1.0 A	87 $\pm$ 7 A	9.6 $\pm$ 0.3 AB

Table 8.7. Effects of length of interval between exposures to ethylene at 250 ppb on reproductive characters in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 6.

Length of intervals	Number of heads	Weight of heads (g)	Number of seeds	Weight per 1000 seeds (g)
control	52 $\pm$ 6 A	17.8 $\pm$ 3.2 A	421 $\pm$ 80 A	25.2 $\pm$ 1.2 A
0 days	60 $\pm$ 6 A	13.9 $\pm$ 1.3 A	241 $\pm$ 27 B	22.8 $\pm$ 0.8 A
1 day	66 $\pm$ 4 A	11.7 $\pm$ 1.3 A	177 $\pm$ 26 B	23.7 $\pm$ 0.4 A
3 days	46 $\pm$ 6 A	14.2 $\pm$ 2.5 A	300 $\pm$ 52 AB	22.4 $\pm$ 1.5 A
5 days	52 $\pm$ 11 A	13.2 $\pm$ 3.2 A	262 $\pm$ 78 AB	20.6 $\pm$ 2.6 A

Table 8.8. Effects of length of interval between exposures to ethylene at 250 ppb on seed quality in barley cv. Harrington. Ethylene exposures were centered around the spike emerging stage. Values are means  $\pm$  S.E. Values in the same column with no letters in common are significantly different at  $P \geq 0.05$ . N = 6.

Length of intervals	% Protein per DW	% Calcium per DW	% Phosphorus per DW
control	34.8 $\pm$ 0.7 <b>A</b>	0.23 $\pm$ 0.01 <b>A</b>	0.75 $\pm$ 0.01 <b>A</b>
0 days	34.6 $\pm$ 0.8 <b>A</b>	0.23 $\pm$ 0.00 <b>A</b>	0.76 $\pm$ 0.01 <b>A</b>
1 day	36.1 $\pm$ 1.1 <b>A</b>	0.22 $\pm$ 0.01 <b>A</b>	0.74 $\pm$ 0.02 <b>A</b>
3 days	35.3 $\pm$ 0.6 <b>A</b>	0.24 $\pm$ 0.00 <b>A</b>	0.76 $\pm$ 0.02 <b>A</b>
5 days	36.1 $\pm$ 0.4 <b>A</b>	0.23 $\pm$ 0.01 <b>A</b>	0.78 $\pm$ 0.03 <b>A</b>

## **9. DOSE RESPONSE FUNCTIONS**

### **9.1 Introduction**

Thus far, the analyses of results made use of the Analysis of Variance, of regression of data against dose expressed as the product of concentration of ethylene and duration of exposure and of plotting various plant parameters against ethylene concentration or duration of exposure. The purpose of this section is to investigate methods of integrating the data generated in this project in order to assist in the understanding of crop responses to ethylene exposure. This section focuses on the analysis of the results from continuous exposure experiments, i.e. short- and long-term exposure experiments. Attempts were made to describe the response of crops to both short- and long-term exposures to ethylene through the use of dose functions based on ethylene concentration and duration of exposure and the incorporation of semi-empirical parameters into the dose function. This led to the derivation of the log-log dose-response function, which was then compared to the Log Sum Dose function developed by Randall D. Jones (pers. comm.) to describe the response of petunia to ethylene exposure.

This discussion will also serve to compare the results from short- and long-term exposure experiments with data used for the development of the interim Alberta Ethylene Guideline (Alberta Environment 1997) and to assess the suitability of using the Log Sum Dose function developed by Randall D. Jones (pers. comm.) of the Ontario Ministry of Environment and Energy, and the log-log dose-response function developed through this study (Appendix III) to describe the responses of Alberta crops to ethylene exposures.

## 9.2 Comparing the Results with the Data Used for the Development of the Alberta Ethylene Guideline (Interim)

In 1997, Alberta Environment conducted an extensive literature review and developed the Alberta Ambient Air Ethylene Guidelines (Interim). The interim guideline states:

Interim Ethylene Guideline	Averaging Period	Daytime Application Period	Seasonal Application Period
104.3 ppb (120 $\mu\text{g m}^{-3}$ )	6 hours	0300 to 2200 hours	May 1 to September 30, inclusive
43.5 ppb (50 $\mu\text{g m}^{-3}$ )	30 days	0300 to 2200 hours	May 1 to September 30, inclusive

To derive and validate the interim guideline, Alberta Environment (1997) selected and compiled two sets of data that describe effects of short-term and long-term ethylene exposure on vegetation. They chose these data based on their relevance to crop production and the lowest concentration and the shortest duration at which a significant effect was observed. With permission from the Alberta Environment, these two figures have been redrawn in the present document (Figs. 9.1 and 9.2). To ease comparisons with data from the present study, data were converted from  $\mu\text{g m}^{-3}$  to ppb, and the results generated through this study were added to the figures. Data from the literature represent instances where exposure of plants to ethylene at 1000 ppb or less caused effects. The short-term experiments (Section 3.0) indicated that no significant effect was observed when three crop species were exposed to ethylene at concentrations up to 1200 ppb for 12 hours. Therefore, according to these findings, exposure of crops to a level of 104.3 ppb ethylene for 6 hours, as setout in the interim guideline, would not have an adverse effect on barley, field pea and canola (Fig. 9.1).

A significant reduction in yield was observed when field pea and canola were exposed to ethylene at concentrations above 100 ppb for 16 days and 50 ppb for 31 days, respectively. Both of these exposures are above the interim guideline and it therefore appears that the interim guideline would be protective of both field pea and canola (Fig. 9.2). However, a significant yield reduction was observed in barley at a concentration of 30 ppb after 14 days of exposure; an exposure below the threshold setout in the interim guideline. This suggests that the interim

guideline may not be protective of this cultivar of barley if the exposure occurs at the sensitive growth stages.

### 9.3 Assessing Ethylene Impacts Using the Log Sum Dose Function

Randall D. Jones (pers. comm.) developed a sum of the log of the average hourly concentration, or a Log Sum Dose (LSD) function to describe the effects of ethylene on petunia flowers. It has been extensively used in the province of Ontario to assess ethylene impact on vegetation. It states:

$$[9-1] \quad \text{Dose} = \sum_{t=0}^{t=x} \log(\bar{X}_{[\text{ethylene}],\text{hourly}})$$

where  $\bar{X}_{[\text{ethylene}],\text{hourly}}$  is the average ethylene concentration in one hour period, or for continuous exposure, it becomes

$$[9-2] \quad \text{Dose} = \log[\text{ethylene}] * \text{duration}.$$

The relative yield (yield expressed as a percent of control) of barley from the short- and long-term experiments plotted against the LSD function is shown in Fig. 9.3. It appears that the Log Sum Dose function does not describe the response of barley to ethylene exposure well for two reasons. It is very difficult to identify the threshold value through the response curve. The threshold dose value is 12.4 according to the LSD function for petunia, while no significant reduction in yield of barley was observed when plants were exposed to an ethylene concentration of 80 ppb for 23 hours (a dose of 45.2 using the LSD function) as seen in our experiment using a demonstration pattern. Thus the LSD does not reflect the sensitivity of barley to ethylene exposure. Secondly, the LSD does not explain the experimental data from two long-term experiments as indicated in Fig. 9.3. Because the function emphasizes the exposure duration, it appears that the yield response to the 14-day exposure is greater than that for the 26-day exposure. Thus, two different response curves were generated for barley rather than a single curve. Abeles et al. (1992) indicated that the dose response function is influenced both by the length of exposure to ethylene and its concentration and that the relationship

between concentration and exposure time is species-dependent. The dose function developed based on the petunia describes the effects of ethylene on petunia well but does not appear to describe well the effects of ethylene on the yield of barley, field pea and canola.

#### 9.4 Assessing Ethylene Impacts Using the Log-log Dose-response Function

We have developed a log-log dose-response function for barley, field pea and canola (Appendix III). It is based on the assumptions that: a) ethylene enters plant tissues through diffusion to the point of action to induce an effect; b) a minimum concentration at the point of action is required to induce an effect; c) ethylene-induced effects on the plant are a function of both concentration and exposure duration; d) there is a time lag before an effect on the plant occurs at any given external ethylene concentration; e) the lag period is inversely related to ethylene concentration in the air.

##### 9.4.1. The Log-log Dose-response Function

The dose in the log-log-response function is defined as

$$[9-3] \quad D = (C_{air} - C_0) \int_0^t \left( 1 - erf\left(\sqrt{\frac{a}{t}}\right) \right) dt = (C_{air} - C_0) f(t)$$

where

$$[9-4] \quad f(t) = \int_0^t \left( 1 - erf\left(\sqrt{\frac{a}{t}}\right) \right) dt$$

$C_{air}$  is the ethylene concentration in air,  $C_0$  is a minimum ethylene concentration below which no response is expected,  $t$  is the exposure duration. The parameter,  $a$ , has a unit of time and expresses the finite lag time discussed in our assumption.

The log-log-response function is:

$$[9-5] \quad \log \frac{Y}{Y_0} = -\left(\log \frac{D_k}{D}\right)^n$$

where  $Y/Y_0$  is the relative yield,  $D$  is the dose,  $D_k$  is the dose where the yield  $Y = 0$  and  $n$  is a dimensionless "shape" factor. It describes the sensitivity of plants to doses less than  $D_k$ .

A threshold dose corresponding to minimum relative yield loss,  $\tau$ , is related n and  $D_k$

$$[9-6] \quad D_\tau = D_k 10^{-(\tau/2.303)^{-1/n}}.$$

Experimental yield data from short- and long-term exposures are plotted as a function of dose, calculated according to equation [9-3] for barley and for field pea and canola (Fig. 9.4 and 9.5). The data for field pea and canola followed a similar trend, suggesting that the sensitivities of these two crops to ethylene exposure are similar.

The data in Figs. 9.4 and 9.5 were fitted to the equation [9-5] using a least-squares technique. For barley, the parameter values that best describe the data are  $n = 2.5$  and  $\log D_k = 4.2$ . At  $\log(Dose) < 1$ , there is no apparent decrease in yield. The coefficient of variance for yield data in these experiments is 0.25. If one assumes that the average yield loss threshold equals to 0.25, the threshold dose is, according to equation [9-3],  $\log(D_\tau) = 1.79$ . If, however, one requires the expected average yield loss to be less than 10%, the threshold dose becomes  $\log(D_\tau) = 0.69$ . For pea and canola, the best parameter values are  $n = 5.0$  and  $\log[D_k] = 4.9$ . The threshold doses corresponding to 10 and 25% expected average yield loss are  $\log(D_\tau) = 3.13$  and  $\log(D_\tau) = 3.40$ , respectively. This again suggests that barley cv. *Harrington* is more sensitive to ethylene exposure than field pea cv. *Carrera* and canola cv. *Quantum*.

The difference in the parameter n for barley and for field pea and canola is worth noticing. The n value for barley is considerably smaller than that for field pea and canola. This means that barley is more sensitive to ethylene exposure at doses less than  $D_k$ . Barley starts to show responses to ethylene at lower doses than field pea and canola and as dose increases toward  $D_k$ , yield decreases more gradually toward 0. For field pea and canola,  $D_k$  is higher. The response to ethylene exposure starts at doses very close to  $D_k$  and as dose increases further toward  $D_k$ , yield decreases much more quickly towards 0 than in the case of barley.

#### 9.4.2. Implications

Several factors are important in the log-log-response function. First is the minimum concentration. For this study, the minimum concentration is chosen as the background concentration of 10 ppb. This value is close to the 12  $\mu\text{g}/\text{m}^3$  used by Randall D. Jones (pers.

comm.) and is consistent with other values given as background in the literature (Reid and Watson 1981; Abeles et. al. 1992). The second is the finite time lag, or minimum exposure time below which response is drastically reduced. This is accounted for by the definition of the time function. The advantage of using a diffusion-based time function over the use of a definite minimum time of exposure is as follows. If a definite minimum time is used, then it is stating that there is no response at exposure times below the minimum regardless of concentration. The diffusion based time function, on the other hand, predicts that at exposure times below the minimum, the response is reduced as exposure time decreases. A response would still be possible if the concentration is high enough. This seems a more realistic assumption. The third important feature is the existence of a maximum dose,  $D_k$ , at which 100% of the yield is lost. At doses less than  $D_k$ , the yield decreases with increasing dose. The fourth important feature of the function is the definition of a minimum, or threshold dose,  $D_t$ . The threshold dose is defined as a function of threshold response or threshold relative to yield loss. If one assumes that the maximum yield loss must be < 10%, the threshold dose will be considerably less than if one assumes a 25% maximum yield loss. The values of 10% and 25% of yield loss were chosen based on the range of the minimum detection limits for the experiments conducted in this study. In other words, if the yield loss is less than 10%, the probability of an experiment to detect existing differences is very low and if the yield loss is high than 25%, the probability of an experiment to detect the differences is very high. The maximum yield loss that could be allowed in deciding the threshold dose must be determined by analyzing the variability of yield and the detection limits of particular experiments. For this report, threshold dose corresponding to 10% and 25% yield losses were calculated.

The distinction must be made between expected response and response that can be observed in a particular experiment. The ability of an experiment to detect existing differences, in this case, a yield reduction, is called the power of the experiment. It is a function of the magnitude of expected response, i.e. yield reduction, at the particular dose, and the precision of the measurements (experimental error). Failure to detect a response when one exists is called the type II error in statistics. The probability of this error decreases as the expected response increases, and as the precision of the experiment increases.

## **9.5 Conclusions**

The data generated through this study are consistent with the data used by Alberta Environment (1997) in the development of the interim guideline for Alberta. The log-log dose-response function fits our data well, compared to the LSD function developed by Randall D. Jones, OMEE for petunia. The analysis using the log-log dose-response function revealed that the threshold doses corresponding to 10 and 25% expected average loss in yield are  $\text{Log}(D_{\tau}) = 0.69$  and  $\text{Log}(D_{\tau}) = 1.79$ , respectively, giving that the coefficient of variance for these data is 0.25. For field pea and canola, the threshold doses corresponding to 10 and 25% expected average yield loss are  $\text{Log}(D_{\tau}) = 3.13$  and  $\text{Log}(D_{\tau}) = 3.40$ , respectively. It is concluded that barley cv. Harrington is more sensitive to ethylene exposure than field pea cv. Carrera and canola cv. Quantum.

According to the log-log dose-response function, the interim Alberta Air Quality Ethylene guidelines of both the ethylene level at 104.3 ppb ( $120 \mu\text{g m}^{-3}$ ) for 6 hours exposure and at 43.5 ppb ( $50 \mu\text{g m}^{-3}$ ) for 30 days exposure resulted in the doses of 0.13 and 2.91 (in log), respectively. Both values are lower than the threshold dose ( $\text{log}(D_{\tau})$ ) value for field pea/canola corresponding to 10% expected average yield loss. However, the dose value of 2.91 from the interim guideline of ethylene concentration of  $50 \mu\text{g m}^{-3}$  for a long-term exposure is higher than the threshold value,  $\text{log}(D_{\tau})$ , of 1.79 corresponding to 25% expected yield loss for barley. This indicated that a significant yield loss, at least 25% yield reduction, for barley would be detected under ethylene level of  $50 \mu\text{g m}^{-3}$  for 30 days, at its sensitive growth stages.

The analysis also indicated that the response of crops to ethylene exposure is a function of concentration and exposure duration and that the expression of this function is species-specific, maybe parameter-specific as well. Therefore, the approach used to develop the log-log dose-response function can be adopted to assess other plant species but it requires validation before the function itself can be used.

## **9.6 Future Considerations**

A significant amount of information regarding the effects of ethylene on crops of Alberta was generated through this study with the aim of providing scientific information for the

development of ambient air guidelines for ethylene and for site-specific risk assessments. If need be, knowledge of the effects of ethylene on crops could be further enhanced. This could be achieved by considering the following:

- A set of short-term exposures ranging from 24 to 72 hours with concentrations ranging from 50 to 250 ppb would be useful to verify the predictability of the log-log dose-response function developed through this project.
- The response of plants to ethylene is species- and growth stage-specific. For this study the most common species and cultivars grown around ethylene production facilities were selected. In a previous study (Archambault and Li 1999) the most sensitive cultivars and growth stages were determined for use in detailed dose response studies with the expectation that results from this study would be applicable to the most common species currently grown in Alberta. These results may or may not apply to future species and cultivars.
- The experiments in this study were conducted in greenhouse and growth chamber conditions. Often plants are more sensitive under these conditions. Therefore the information more likely falls on the conservative side. Confirmation of these research findings, which would provide a more realistic estimation of the response of crops to ethylene, should be conducted under field conditions. With the knowledge we gained through this study, a cost-effective field program could be conducted.

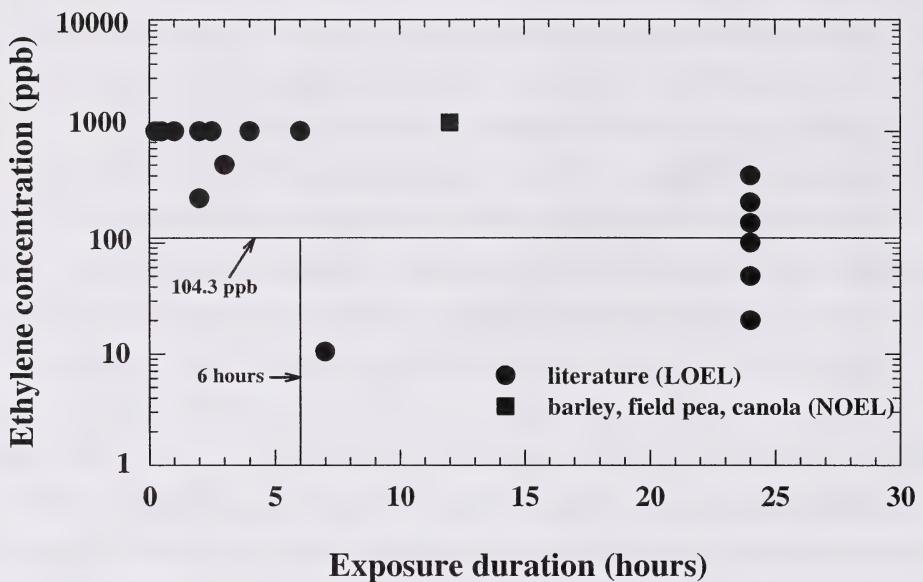


Figure 9.1. Comparison of the data generated from this study with the data used in development of the Alberta Air Quality Ethylene Guideline (interim) for short-term ethylene exposure. Data from the literature represent instances where exposure of plants to ethylene at 1000 ppb or less caused effects (LOEL = least observable effects levels). The data for barley, field pea and canola represents results of short-term exposures to ethylene where no effects were observed (NOEL = no observed effects level). (Adopted from Alberta Environment 1997)

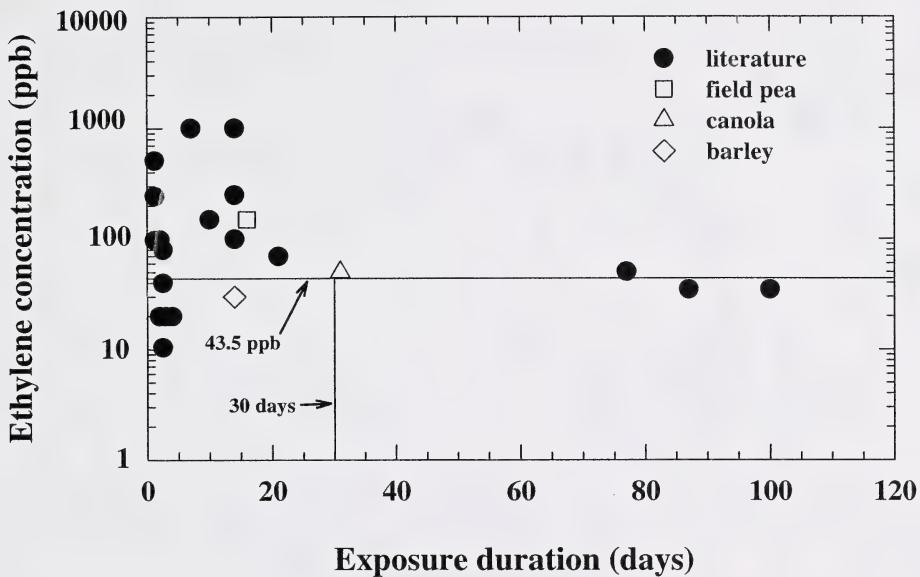


Figure 9.2. Comparison of the data generated from this study with the data used in development of the Alberta Air Quality Ethylene Guideline (interim) for long-term ethylene exposure. Data from the literature represent instances where exposure of plants to ethylene at 1000 ppb or less caused effects. (Adopted from Alberta Environment 1997)

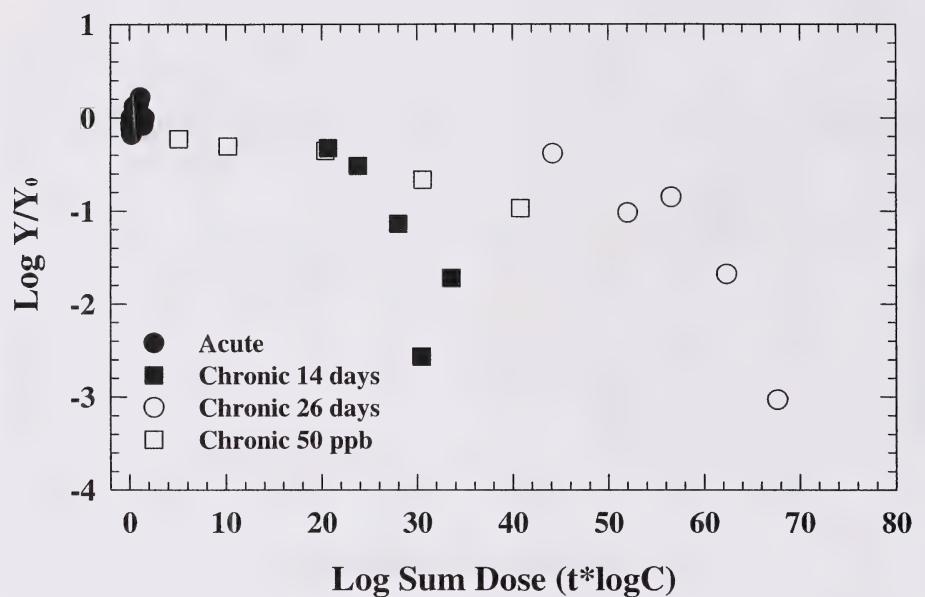


Figure 9.3. Relative yield of barley cv. Harrington is plotted as a function of the Log Sum Dose (LSD). Data from short- and long-term ethylene exposure experiments were pooled.

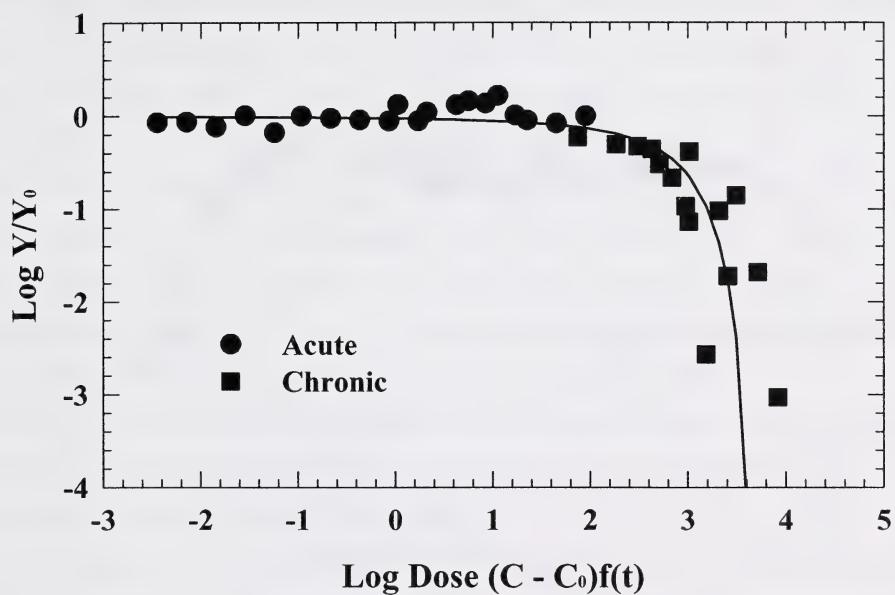


Figure 9.4. Relative yield of barley cv. Harrington is plotted as a function of the log dose. Data from short- and long-term ethylene exposure experiments were pooled.

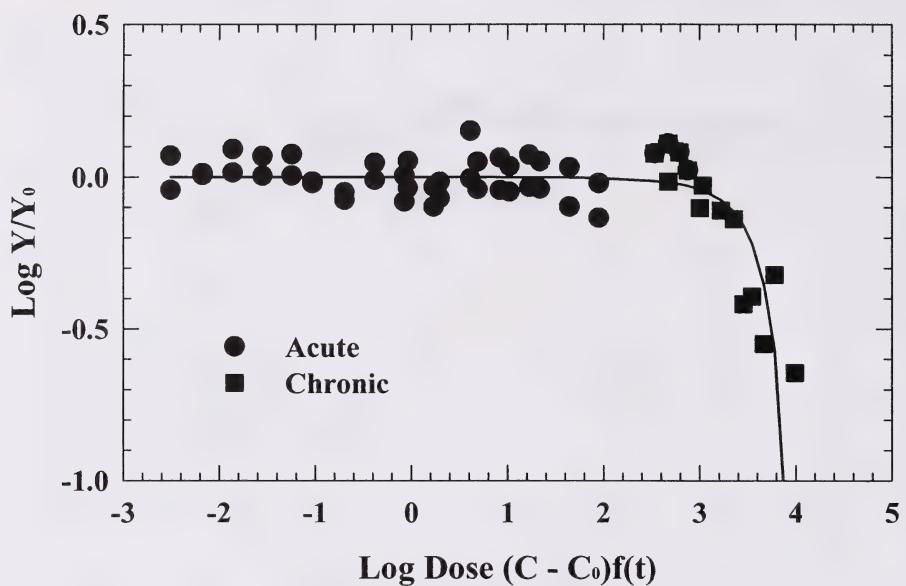


Figure 9.5. Relative yields of field pea cv. Carrera and canola cv. Quantum are plotted as a function of the log dose. Data from short- and long-term ethylene exposure experiments were pooled.

## **10. GENERAL CONCLUSIONS**

The goals of the Alberta Ethylene Crop Research Project were to provide scientific information to Alberta Environment for further development of ambient air quality guidelines for ethylene and to provide the petrochemical industry and communities adjacent to petrochemical facilities with scientific information for site-specific risk assessment. To achieve these goals, the responses of cultivars of agricultural crops of interest to Alberta producers to ethylene exposure were investigated. It was concluded that:

1. No significant effects of short-term exposure to ethylene, up to 1200 ppb for 12 hours, were observed on the parameters measured for barley, field pea and canola. Similarly, no effects of short-term exposures to ethylene, up to 1200 ppb for 12 hours, were observed on seed germination, seedling vigour, growth in the rapid growth phase and seedling marketability in lodgepole pine and white spruce.
2. Several symptoms of ethylene on vegetative and reproductive characters were observed in the three crop species studied. Ethylene symptoms appeared earliest in field pea and symptoms were most noticeable (Appendix II).
3. The response of plants to long-term ethylene exposure depends on both concentration and length of exposure. Minimum ethylene concentrations and length of exposure required to cause an effect are species-specific. While field pea did not respond to long-term exposures to ethylene at 50 ppb, barley yields were reduced significantly when plants were exposed to 50 ppb ethylene for 3 days.
4. Significant reductions in yield of field pea were observed in plants exposed to ethylene at concentrations above 100 ppb for 16 days. Complete recovery of yield in field pea was observed following exposure to ethylene. Barley and canola did not appear to have the recovery abilities of field pea.
5. Barley cv. Harrington is more sensitive to ethylene exposure than field pea cv. Carrera and canola cv. Quantum.
6. Time of day of exposure is an important factor in determining the relative sensitivities of plants to ethylene. Barley was most sensitive to ethylene between 10:00 a.m. to 4:00 p.m.
7. Exposure of barley plants at the most sensitive stages of development to a demonstration pattern generated from ambient air monitoring data from a position adjacent to a petrochemical facility had no significant effect on yield.

8. Intermittent exposure experiments indicated that exposure of barley to 5 intermittent exposures of ethylene at 250 ppb caused a reduction in yield. Intervals between exposures as long as 5 days were not sufficient to allow for complete recovery.
9. Analysis using the log-log dose-response function revealed that the dose derived from the 6-hour interim Alberta Air Quality Ethylene guideline ( $120 \mu\text{g m}^{-3}$  or 104 ppb) was lower than the threshold values for 10% decrease in yield of field pea, canola and barley. The dose derived from the 30-day guideline ( $50 \mu\text{g m}^{-3}$  or 44 ppb) was lower than the threshold values for 10% decrease in yield of field pea and canola but higher than the threshold value for barley.

This study provides scientific information that can be used by Alberta Environment to further develop ambient air quality guidelines for ethylene and for the petrochemical industry and communities adjacent to petrochemical facilities to conduct site-specific risk assessments.

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## **APPENDIX I. DEVELOPMENT AND EVALUATION OF EXPERIMENTAL PROCEDURES OF SHORT-TERM EXPOSURE TO ETHYLENE.**

### **Summary**

A number of preliminary trials were conducted to develop and test short-term exposure protocols prior to conducting the formal trials described in detail in the main portion of this report. This appendix serves as a summary of the results of those preliminary trials.

Field pea and canola trials were designed as those formal trials reported herein. Barley trials were designed to test for differences in ethylene effects between day and night exposures using a 6h exposure from either 9 a.m. to 3 p.m. or from 11:00 p.m. to 5:00 a.m. The ethylene concentrations are reported as average gas chromatograph (GC) measurements (Table I-1). All plant assessments were conducted as reported here and/or in Archambault and Li (1999).

For field pea cv. *Carrera*, no effects of ethylene on plant heights were found (Table 2). Statistically significant effects of ethylene treatment on above-ground biomass were found when plants were treated for 1.5 hours at 345 ppb (increase) and for 6 hours at 70 ppb (decrease). Increased stem thickness was only found when plants were treated with ethylene for 12 hours at 1050 ppb (Table I-2).

Treatment with ethylene caused a decrease of 25% in the number of yellow pods per pot when plants were treated for 1.5 hours at 630 ppb and of 23% when plants were treated for 6 hours at 1140 ppb (Table I-3). While the number of green pods per pot was unaffected by treatment with ethylene, the total number of pods per pot was decreased when plants were treated for 1.5 hours at 630 ppb and for 6 hours at 70 ppb. Total pod weights were only significantly decreased when plants were treated for 12 hours at 160 ppb (Table I-3).

No effects on seed numbers per pot were found (Table I-4). Treatment effects on seed weights per pot were only found when plants were treated for 12 hours at concentrations of 160 ppb and 395 ppb with decreases of 29 and 22%, respectively. Treatment effects on weight per thousand seeds were observed when plants were treated with ethylene for 1.5 hours at 630 ppb (increase) and for 12 hours at 160 ppb (decrease). Reductions in energy content of seeds were observed

when plants were treated for 6 hours at 70 ppb, 295 ppb and 1140 ppb. Percent nitrogen increased when plants were treated for 6 hours at 295 ppb and 1140 ppb (Table I-4). No significant correlations were found when biomass, seed weight and number of pods per pot were plotted against ethylene dose (concentration x duration) with  $R^2$  values of 0.0051, 0.0136 and 0.0094, respectively (Figure I-1).

For barley cv. Harrington, height was significantly increased when plants were exposed to ethylene at a concentration of 820 ppb in the daytime (Table I-5). Above-ground biomass was suppressed when plants were exposed to 90 ppb and 180 ppb ethylene at nighttime. While stem thickness and number of tillers were unaffected by ethylene treatment in the daytime and nighttime, internode length was significantly increased when plants were treated with 500 ppb ethylene at nighttime (Table I-5).

Ethylene treatment in the daytime and nighttime had no effect on number of heads or weight of heads per pot (Table I-6). The number and weight of seeds per pot were both suppressed by approximately 34% when plants were exposed to 90 ppb ethylene at nighttime. No effects of ethylene were found on weight per thousand seeds nor on protein, calcium, phosphorus and energy contents of seeds (Tables I-6 and I-7).

For canola cv. Quantum, exposure to ethylene had no effect on height, above-ground biomass, pod weights, number of seeds, weight of seeds per pot at any of the dosages used (Table I-8). Root weights were decreased when plants were exposed to 55 ppb, 75 ppb and 100 ppb ethylene for 12 hours. Weight per thousand seeds values were depressed by 17% when plants were treated with 100 ppb ethylene for 12 hours (Table I-8). No significant correlations were found when biomass and seed weight per pot were plotted against ethylene dose (concentration x duration) with  $R^2$  values of 0.093 and 0.044, respectively (Figure I-2).

Few effects of short-term exposure to ethylene were found with no clear dose response in any of the parameters measured. Overall, the experimental protocol was adequate for testing for effects of short-term exposure to ethylene in all three crop species selected. As a whole, the exposure system performed well but failed to consistently produce exposure levels within 10%

of the target concentrations. Over the progression of the preliminary experimental period, the causes of the exposure system failures were discovered and rectified. Ethylene concentrations for short-term exposure in formal trials were always within 10% of target.

Table I-1. Ethylene concentrations for preliminary short-term exposure experiments using field pea and canola.

Duration (hours)	Target conc. (ppb)	GC meas. (av. in ppb)	Target conc. (ppb)	GC meas. (av. in ppb)
Field pea			Canola	
1.5	10	3	10	9
	75	80	75	55
	150	150	150	140
	300	345	300	290
	600	630	600	600
	1200	1000	1200	1180
3	10	12	10	8
	75	100	75	90
	150	155	150	165
	300	345	300	320
	600	650	600	680
	1200	1250	1200	1260
6	10	9	10	8
	75	70	75	90
	150	165	150	175
	300	295	300	275
	600	295	600	605
	1200	1140	1200	1250
12	10	10	10	9
	75	70	75	55
	150	160	150	75
	300	395	300	100
	600	700	600	450
	1200	1050	1200	940

Table I-2. Vegetative parameters for field pea cv. Carrera. Plants were treated with ethylene at the flat pod stage for 1.5, 3, 6 or 12 hours. Numbers represent means  $\pm$  S.E. N = 5.

Length of exp. (h)	Eth. conc. (ppb)	Plant height (cm)	Vegetative A-G biomass (g)	Stem thick. (mm)
1.5	3	69.2 $\pm$ 2.6	10.9 $\pm$ 0.8	2.29 $\pm$ 0.10
	80	71.4 $\pm$ 3.9	13.1 $\pm$ 0.7	2.36 $\pm$ 0.20
	150	65.5 $\pm$ 3.2	10.2 $\pm$ 1.06	2.23 $\pm$ 0.17
	345	75.9 $\pm$ 2.2	13.4 $\pm$ 0.6	2.11 $\pm$ 0.20
	630	68.4 $\pm$ 2.5	9.9 $\pm$ 1.1	2.19 $\pm$ 0.18
	1000	73.1 $\pm$ 1.9	12.4 $\pm$ 0.6	2.54 $\pm$ 0.06
3	12	71.0 $\pm$ 3.9	11.0 $\pm$ 1.1	2.27 $\pm$ 0.07
	100	78.9 $\pm$ 2.1	11.9 $\pm$ 0.5	2.08 $\pm$ 0.12
	155	68.4 $\pm$ 3.3	9.4 $\pm$ 1.4	2.24 $\pm$ 0.17
	345	78.4 $\pm$ 2.3	11.8 $\pm$ 0.3	2.09 $\pm$ 0.17
	650	76.0 $\pm$ 2.4	10.5 $\pm$ 0.6	2.28 $\pm$ 0.08
	1250	78.9 $\pm$ 3.9	10.1 $\pm$ 0.6	2.10 $\pm$ 0.14

Table I-2 continued...

Length of exp. (h)	Eth. conc. (ppb)	Plant height (cm)	A-G biomass (g)	Stem thick. (mm)
6	9	73.2 $\pm$ 3.2	11.3 $\pm$ 1.0	2.47 $\pm$ 0.13
	70	71.1 $\pm$ 5.7	8.2 $\pm$ 0.7	2.37 $\pm$ 0.08
	165	78.7 $\pm$ 1.5	12.2 $\pm$ 0.7	2.47 $\pm$ 0.17
	295	71.4 $\pm$ 4.1	9.8 $\pm$ 1.4	2.28 $\pm$ 0.09
	295	69.5 $\pm$ 5.1	9.5 $\pm$ 1.0	2.22 $\pm$ 0.12
	1140	69.9 $\pm$ 3.4	8.6 $\pm$ 0.6	2.28 $\pm$ 0.21
12	10	71.0 $\pm$ 2.7	11.5 $\pm$ 0.8	2.11 $\pm$ 0.16
	70	68.2 $\pm$ 2.6	10.3 $\pm$ 1.4	2.32 $\pm$ 0.07
	160	63.3 $\pm$ 4.4	10.4 $\pm$ 1.1	1.98 $\pm$ 0.17
	395	65.5 $\pm$ 1.7	10.1 $\pm$ 1.0	2.27 $\pm$ 0.03
	700	69.7 $\pm$ 4.6	11.5 $\pm$ 1.3	2.37 $\pm$ 0.19
	1050	75.3 $\pm$ 1.6	13.2 $\pm$ 0.6	2.63 $\pm$ 0.08

Table I-3. Pod data for field pea cv. Carrera. Plants were treated with ethylene at the flat pod stage for 1.5, 3, 6 or 12 hours. Numbers represent means  $\pm$  S.E. N = 5.

Length of exp. (h)	Eth. conc. (ppb)	Number of yellow pods	Number of green pods	Total # of pods	Total pod weight (g)
1.5	3	16 $\pm$ 1	0 $\pm$ 0	16 $\pm$ 1	11.7 $\pm$ 0.9
	80	16 $\pm$ 1	1 $\pm$ 0	17 $\pm$ 1	13.3 $\pm$ 1.0
	150	12 $\pm$ 2	2 $\pm$ 2	14 $\pm$ 2	11.8 $\pm$ 1.8
	345	13 $\pm$ 0	1 $\pm$ 0	14 $\pm$ 0	14.0 $\pm$ 1.0
	630	12 $\pm$ 1	0 $\pm$ 0	12 $\pm$ 1	13.4 $\pm$ 1.3
	1000	14 $\pm$ 0	0 $\pm$ 0	14 $\pm$ 0	14.3 $\pm$ 0.9
3	12	12 $\pm$ 1	1 $\pm$ 0	13 $\pm$ 1	13.4 $\pm$ 1.1
	100	13 $\pm$ 1	2 $\pm$ 1	15 $\pm$ 2	14.9 $\pm$ 1.7
	155	11 $\pm$ 2	1 $\pm$ 1	12 $\pm$ 1	14.4 $\pm$ 1.4
	345	14 $\pm$ 1	2 $\pm$ 0	16 $\pm$ 1	16.5 $\pm$ 1.4
	650	13 $\pm$ 1	1 $\pm$ 1	14 $\pm$ 1	16.5 $\pm$ 0.9
	1250	12 $\pm$ 1	1 $\pm$ 3	13 $\pm$ 1	14.7 $\pm$ 0.9

Table I-3 continued...

Length of exp. (h)	Eth. conc. (ppb)	Number of yellow pods	Number of green pods	Total # of pods	Total pod weight (g)
6	9	13 $\pm$ 1	2 $\pm$ 1	15 $\pm$ 1	15.5 $\pm$ 0.9
	70	11 $\pm$ 1	0 $\pm$ 0	11 $\pm$ 1	11.9 $\pm$ 1.0
	165	13 $\pm$ 0	3 $\pm$ 2	16 $\pm$ 2	14.9 $\pm$ 1.0
	295	11 $\pm$ 1	1 $\pm$ 1	12 $\pm$ 1	13.1 $\pm$ 1.2
	295	12 $\pm$ 1	1 $\pm$ 1	13 $\pm$ 1	14.2 $\pm$ 2.1
	1140	10 $\pm$ 1	1 $\pm$ 1	11 $\pm$ 0	13.4 $\pm$ 1.2
12	10	14 $\pm$ 1	1 $\pm$ 0	15 $\pm$ 1	14.1 $\pm$ 0.9
	70	13 $\pm$ 1	2 $\pm$ 2	15 $\pm$ 2	13.6 $\pm$ 0.9
	160	12 $\pm$ 2	1 $\pm$ 1	13 $\pm$ 1	10.5 $\pm$ 1.5
	395	13 $\pm$ 1	1 $\pm$ 0	14 $\pm$ 1	11.2 $\pm$ 0.6
	700	15 $\pm$ 1	1 $\pm$ 0	16 $\pm$ 2	12.5 $\pm$ 1.0
	1050	18 $\pm$ 1	0 $\pm$ 0	18 $\pm$ 1	14.6 $\pm$ 0.5

Table I-4. Seed data for field pea cv. Carrera. Plants were treated with ethylene at the flat pod stage for 1.5, 3, 6 or 12 hours. Numbers represent means  $\pm$  S.E. N = 5.

Length of exp. (h)	Eth. conc. (ppb)	Number of seeds	Weight of seeds (g)	Weight/1000 seeds (g)	Calories per gram	Nitrogen %
1.5	3	63 $\pm$ 5	9.3 $\pm$ 0.7	149.7 $\pm$ 12.8	4200 $\pm$ 6	4.90 $\pm$ 0.12
	80	75 $\pm$ 6	10.6 $\pm$ 0.9	141.3 $\pm$ 9.9	4193 $\pm$ 9	4.98 $\pm$ 0.17
	150	58 $\pm$ 7	9.4 $\pm$ 1.5	159.9 $\pm$ 17.4	4211 $\pm$ 6	4.96 $\pm$ 0.27
	345	68 $\pm$ 3	11.4 $\pm$ 1.0	167.2 $\pm$ 12.0	4186 $\pm$ 4	5.13 $\pm$ 0.30
	630	59 $\pm$ 6	11.2 $\pm$ 1.1	193.2 $\pm$ 10.5	4183 $\pm$ 7	4.54 $\pm$ 0.14
	1000	66 $\pm$ 1	11.8 $\pm$ 0.9	180.2 $\pm$ 14.9	4208 $\pm$ 11	4.79 $\pm$ 0.14
3	12	55 $\pm$ 3	11.4 $\pm$ 0.9	204.8 $\pm$ 11.0	4245 $\pm$ 8	4.27 $\pm$ 0.07
	100	60 $\pm$ 5	12.7 $\pm$ 1.5	208.3 $\pm$ 11.8	4246 $\pm$ 5	4.23 $\pm$ 0.14
	155	55 $\pm$ 8	12.3 $\pm$ 1.2	228.1 $\pm$ 10.6	4256 $\pm$ 5	4.19 $\pm$ 0.08
	345	68 $\pm$ 3	13.9 $\pm$ 1.2	222.4 $\pm$ 11.6	4256 $\pm$ 4	4.29 $\pm$ 0.06
	650	63 $\pm$ 6	13.9 $\pm$ 0.8	224.8 $\pm$ 12.9	4237 $\pm$ 7	4.32 $\pm$ 0.12
	1250	58 $\pm$ 4	12.6 $\pm$ 0.9	219.9 $\pm$ 13.3	4233 $\pm$ 4	4.28 $\pm$ 0.08

Table I-4 continued...

Length of exp. (h)	Eth. conc. (ppb)	Number of seeds	Weight of seeds (g)	Wt/1000 seeds (g)	Calories per gram	Nitrogen %
6	9	66 $\pm$ 2	13.2 $\pm$ 0.8	201.0 $\pm$ 10.4	4230 $\pm$ 8	4.10 $\pm$ 0.05
	70	52 $\pm$ 4	10.1 $\pm$ 0.8	196.0 $\pm$ 7.4	4191 $\pm$ 5	4.15 $\pm$ 0.08
	165	65 $\pm$ 4	12.5 $\pm$ 1.0	191.3 $\pm$ 11.3	4231 $\pm$ 11	4.37 $\pm$ 0.11
	295	59 $\pm$ 6	1.0 $\pm$ 1.1	187.1 $\pm$ 9.4	4200 $\pm$ 5	4.25 $\pm$ 0.08
	295	61 $\pm$ 6	12.0 $\pm$ 1.9	190.5 $\pm$ 18.2	4199 $\pm$ 6	4.77 $\pm$ 0.28
	1140	55 $\pm$ 4	11.4 $\pm$ 1.0	209.2 $\pm$ 12.0	4187 $\pm$ 9	4.56 $\pm$ 0.13
12	10	67 $\pm$ 3	11.8 $\pm$ 0.8	176.8 $\pm$ 5.7	4237 $\pm$ 6	4.47 $\pm$ 0.14
	70	59 $\pm$ 6	11.3 $\pm$ 0.7	196.2 $\pm$ 9.6	4229 $\pm$ 9	4.30 $\pm$ 0.09
	160	57 $\pm$ 6	8.4 $\pm$ 1.3	143.8 $\pm$ 14.1	4220 $\pm$ 13	4.72 $\pm$ 0.10
	395	57 $\pm$ 5	9.2 $\pm$ 0.5	163.2 $\pm$ 8.9	4231 $\pm$ 4	4.61 $\pm$ 0.12
	700	62 $\pm$ 7	10.3 $\pm$ 0.8	171.5 $\pm$ 18.2	4242 $\pm$ 10	4.52 $\pm$ 0.15
	1050	72 $\pm$ 3	12.0 $\pm$ 0.4	166.9 $\pm$ 5.5	4224 $\pm$ 6	4.46 $\pm$ 0.16

Table I-5. Vegetative parameters for barley cv. Harrington. Plants were treated with ethylene at the spike emerging stage for 6 hours either in the daytime or nighttime. Numbers represent means  $\pm$  S.E. N = 5.

Time of day	Eth. conc. (ppb)	Plant height (cm)	A-G biomass (g)	Stem thick. (mm)	Internode length (cm)	Number of tillers
day	0	86.2 $\pm$ 1.7	103.3 $\pm$ 6.6	3.42 $\pm$ 0.15	5.58 $\pm$ 0.61	38 $\pm$ 4
	65	91.2 $\pm$ 3.2	93.3 $\pm$ 8.1	3.15 $\pm$ 0.20	5.32 $\pm$ 0.28	30 $\pm$ 3
	90	88.4 $\pm$ 1.3	102.1 $\pm$ 8.2	3.54 $\pm$ 0.22	7.44 $\pm$ 0.74	30 $\pm$ 5
	225	91.6 $\pm$ 3.3	96.4 $\pm$ 9.1	2.83 $\pm$ 0.15	7.20 $\pm$ 0.58	31 $\pm$ 3
	450	92.5 $\pm$ 1.2	105.2 $\pm$ 5.4	3.33 $\pm$ 0.23	5.58 $\pm$ 0.34	34 $\pm$ 6
	820	94.8 $\pm$ 2.4	95.5 $\pm$ 4.9	3.64 $\pm$ 0.11	7.36 $\pm$ 0.73	30 $\pm$ 4
night	0	86.6 $\pm$ 1.4	115.0 $\pm$ 3.3	3.40 $\pm$ 0.28	5.80 $\pm$ 0.25	43 $\pm$ 4
	50	90.7 $\pm$ 1.6	110.4 $\pm$ 2.5	3.38 $\pm$ 0.17	7.54 $\pm$ 0.35	42 $\pm$ 6
	90	90.6 $\pm$ 2.4	96.7 $\pm$ 6.1	2.98 $\pm$ 0.11	7.36 $\pm$ 0.57	37 $\pm$ 4
	180	91.8 $\pm$ 2.5	100.4 $\pm$ 4.6	3.19 $\pm$ 0.22	7.36 $\pm$ 0.79	30 $\pm$ 4
	500	90.0 $\pm$ 1.7	112.3 $\pm$ 3.6	3.18 $\pm$ 0.21	7.72 $\pm$ 0.42	46 $\pm$ 7
	750	88.7 $\pm$ 0.9	103.0 $\pm$ 5.8	2.72 $\pm$ 0.25	7.48 $\pm$ 0.8	33 $\pm$ 5

Table I-6. Head and seed data for barley cv. Harrington. Plants were treated with ethylene at the spike emerging stage for 6 hours either in the daytime or nighttime. All data are on a per pot (5 plants) basis. Numbers represent means  $\pm$  S.E. N = 5.

Time of day	Eth. conc. (ppb)	Number of heads	Weight of heads (g)	# of seeds	Weight of seeds (g)	Wt/1000 seeds (g)
day	0	124 $\pm$ 7	72.9 $\pm$ 7.2	1400 $\pm$ 166	53.9 $\pm$ 7.3	38.2 $\pm$ 0.9
	65	95 $\pm$ 9	60.3 $\pm$ 7.4	1108 $\pm$ 134	44.2 $\pm$ 7.1	39.1 $\pm$ 2.4
	90	110 $\pm$ 18	57.9 $\pm$ 10.0	1048 $\pm$ 225	38.0 $\pm$ 9.6	34.4 $\pm$ 2.7
	225	99 $\pm$ 8	56.7 $\pm$ 10.0	92.6 $\pm$ 209	38.6 $\pm$ 9.1	39.5 $\pm$ 2.7
	450	109 $\pm$ 8	65.1 $\pm$ 8.2	1190 $\pm$ 137	45.0 $\pm$ 8.4	36.5 $\pm$ 3.5
	820	111 $\pm$ 11	63.0 $\pm$ 5.6	1120 $\pm$ 166	41.4 $\pm$ 7.9	35.7 $\pm$ 2.9
night	0	133 $\pm$ 6	60.1 $\pm$ 1.7	1219 $\pm$ 66	49.4 $\pm$ 2.6	40.6 $\pm$ 0.9
	50	134 $\pm$ 10	64.8 $\pm$ 4.5	1032 $\pm$ 40	43.2 $\pm$ 2.5	41.9 $\pm$ 1.8
	90	114 $\pm$ 19	57.9 $\pm$ 12.5	810 $\pm$ 203	330 $\pm$ 8.4	38.2 $\pm$ 2.8
	180	102 $\pm$ 5	54.4 $\pm$ 3.6	958 $\pm$ 109	35.1 $\pm$ 4.8	36.3 $\pm$ 1.4
	500	145 $\pm$ 6	63.0 $\pm$ 1.1	1058 $\pm$ 19	43.4 $\pm$ 1.4	41.0 $\pm$ 0.8
	750	150 $\pm$ 11	60.8 $\pm$ 4.0	1015 $\pm$ 76	38.8 $\pm$ 3.4	38.2 $\pm$ 1.5

Table I-7. Seed quality data for barley cv. Harrington. Plants were treated with ethylene at the spike emerging stage for 6 hours either in the daytime or nighttime. Numbers represent means  $\pm$  S.E. N = 5.

Time of day	Eth. conc. (ppb)	Protein %	Calcium %	Phosphorus %	Calories per gram
day	0	22.0 $\pm$ 0.5	0.16 $\pm$ 0.01	0.52 $\pm$ 0.01	4245 $\pm$ 9
	50	24.2 $\pm$ 1.3	0.18 $\pm$ 0.02	0.57 $\pm$ 0.02	4246 $\pm$ 9
	100	22.5 $\pm$ 0.8	0.15 $\pm$ 0.01	0.53 $\pm$ 0.01	4247 $\pm$ 12
	200	22.8 $\pm$ 0.3	0.16 $\pm$ 0.01	0.54 $\pm$ 0.01	4241 $\pm$ 5
	400	21.6 $\pm$ 0.4	0.14 $\pm$ 0.01	0.52 $\pm$ 0.01	4238 $\pm$ 9
	800	21.9 $\pm$ 0.6	0.16 $\pm$ 0.02	0.51 $\pm$ 0.01	4241 $\pm$ 8
night	0	22.0 $\pm$ 0.6	0.15 $\pm$ 0.00	0.51 $\pm$ 0.01	4241 $\pm$ 7
	50	21.8 $\pm$ 0.7	0.17 $\pm$ 0.02	0.51 $\pm$ 0.01	4244 $\pm$ 8
	100	21.4 $\pm$ 0.9	0.13 $\pm$ 0.00	0.52 $\pm$ 0.01	4248 $\pm$ 11
	200	23.5 $\pm$ 0.5	0.16 $\pm$ 0.01	0.53 $\pm$ 0.01	4268 $\pm$ 6
	400	20.8 $\pm$ 0.5	0.13 $\pm$ 0.00	0.51 $\pm$ 0.01	4253 $\pm$ 11
	800	22.2 $\pm$ 0.2	0.16 $\pm$ 0.00	0.51 $\pm$ 0.00	4247 $\pm$ 7

Table I-8. Biomass, pod and seed data for canola cv. Quantum. Plants were treated with ethylene at the many flowers stage for 1.5, 3, 6 or 12 hours. Numbers represent means  $\pm$  S.E. N = 5.

Length of exp. (h)	Eth. conc. (ppb)	Height (cm)	A-G Vegetative Biomass (g)	Root Wt. (g)	Pod Weights (g)	# of seeds	Seed Weight (g)	Weight per 1000 seeds (g)
1.5	9	114.2 $\pm$ 5.4	28.6 $\pm$ 4.4	4.81 $\pm$ 1.21	38.4 $\pm$ 3.0	6358 $\pm$ 1242	16.7 $\pm$ 2.	2.77 $\pm$ 0.25
	55	105.2 $\pm$ 3.7	22.2 $\pm$ 3.2	4.06 $\pm$ 0.96	30.5 $\pm$ 5.2	4786 $\pm$ 726	13.2 $\pm$ 2.3	2.75 $\pm$ 0.10
	140	117.4 $\pm$ 7.1	34.1 $\pm$ 3.3	6.08 $\pm$ 1.68	43.9 $\pm$ 4.6	7293 $\pm$ 666	18.4 $\pm$ 1.8	2.52 $\pm$ 0.09
	290	113.4 $\pm$ 7.4	33.8 $\pm$ 5.7	10.78 $\pm$ 5.28	40.5 $\pm$ 4.5	6006 $\pm$ 860	16.1 $\pm$ 1.9	2.73 $\pm$ 0.16
	600	114.6 $\pm$ 3.5	28.7 $\pm$ 3.6	4.75 $\pm$ 0.92	41.4 $\pm$ 3.2	7080 $\pm$ 830	17.2 $\pm$ 2.6	2.42 $\pm$ 0.13
	1180	105.4 $\pm$ 6.7	26.2 $\pm$ 4.1	5.33 $\pm$ 1.05	36.3 $\pm$ 3.2	6127 $\pm$ 1300	16.3 $\pm$ 3.5	2.74 $\pm$ 0.16
3	8	121.0 $\pm$ 5.4	27.6 $\pm$ 2.1	5.59 $\pm$ 0.95	32.6 $\pm$ 6.1	4962 $\pm$ 537	12.8 $\pm$ 1.1	2.61 $\pm$ 0.13
	90	114.6 $\pm$ 6.0	25.5 $\pm$ 2.3	5.15 $\pm$ 1.09	32.2 $\pm$ 4.8	5727 $\pm$ 932	13.3 $\pm$ 2.2	2.31 $\pm$ 0.03
	165	127.8 $\pm$ 6.8	32.8 $\pm$ 2.3	5.78 $\pm$ 0.90	34.0 $\pm$ 3.5	5265 $\pm$ 724	12.6 $\pm$ 2.1	2.36 $\pm$ 0.16
	320	121.6 $\pm$ 1.9	28.4 $\pm$ 1.6	5.22 $\pm$ 0.76	34.5 $\pm$ 5.5	6002 $\pm$ 838	13.6 $\pm$ 2.0	2.27 $\pm$ 0.10
	680	111.6 $\pm$ 3.6	30.5 $\pm$ 2.9	5.02 $\pm$ 0.97	25.8 $\pm$ 5.7	3763 $\pm$ 546	9.3 $\pm$ 1.3	2.50 $\pm$ 0.11
	1260	124.8 $\pm$ 2.8	32.5 $\pm$ 2.3	6.86 $\pm$ 1.29	33.1 $\pm$ 7.3	5338 $\pm$ 650	12.4 $\pm$ 1.5	2.33 $\pm$ 0.11

Table I-8 continued

Length of exp. (h)	Eth. conc. (ppb)	Height (cm)	A-G Vegetative Biomass (g)	Root Wt. (g)	Pod Weights (g)	# of seeds	Seed Weight (g)	Weight per 1000 seeds (g)
6	8	108.2 ± 3.0	25.9 ± 2.1	3.78 ± 0.42	32.9 ± 6.3	5603 ± 1500	14.0 ± 3.6	2.53 ± 0.10
	90	107.0 ± 2.7	27.4 ± 3.2	4.81 ± 0.80	38.2 ± 7.2	6299 ± 1175	16.0 ± 3.4	2.53 ± 0.14
	175	102.2 ± 5.6	25.0 ± 2.7	2.93 ± 0.38	25.7 ± 4.4	3766 ± 622	9.9 ± 2.0	2.62 ± 0.13
	275	112.4 ± 3.6	31.0 ± 2.7	4.05 ± 0.45	36.1 ± 3.4	5970 ± 715	14.5 ± 1.7	2.43 ± 0.07
	605	113.4 ± 9.8	24.5 ± 5.6	3.73 ± 0.93	34.4 ± 8.7	5616 ± 1380	14.1 ± 3.4	2.52 ± 0.11
	1250	124.0 ± 4.2	31.1 ± 1.8	5.31 ± 0.97	39.0 ± 3.34	6472 ± 615	15.6 ± 1.6	2.40 ± 0.05
12	9	113.0 ± 3.0	39.2 ± 3.5	8.75 ± 1.17	46.9 ± 4.9	6725 ± 839	20.2 ± 2.3	3.06 ± 0.15
	55	119.0 ± 1.3	33.0 ± 2.3	4.59 ± 0.33	44.7 ± 2.7	6762 ± 491	19.2 ± 1.2	2.84 ± 0.06
	75	116.4 ± 3.8	35.4 ± 2.8	5.92 ± 0.59	49.0 ± 3.2	7456 ± 747	20.1 ± 1.7	2.73 ± 0.15
	100	113.2 ± 3.1	31.5 ± 2.1	4.83 ± 0.42	47.3 ± 5.6	8355 ± 1292	21.1 ± 3.1	2.55 ± 0.12
	450	118.6 ± 4.4	36.4 ± 1.8	6.78 ± 0.77	59.4 ± 1.3	8257 ± 490	25.8 ± 0.9	3.14 ± 0.11
	940	119.6 ± 5.8	35.5 ± 1.2	6.92 ± 1.28	51.1 ± 4.8	8020 ± 971	23.0 ± 2.4	2.92 ± 0.20

## **APPENDIX II. VISUAL SYMPTOMS OF ETHYLENE-INJURED CROPS.**

Photos are available on CD-Rom provided herewith.

### **APPENDIX III. DEVELOPMENT OF A DOSE-RESPONSE EQUATION FOR ETHYLENE EXPOSURE**

#### Considerations in the development of dose-response relationships

In order to induce an effect on growth and function of plants, ethylene must first enter plant tissues, reach the point of action at a sufficient concentration and initiate a cascade of metabolic responses. The time required for these events to occur can be seen as a lag in response. The magnitude of the ethylene-induced effect on the plant is expected to be a function of both the ethylene concentration in the air and the duration of exposure.

After the lag period, plant response increases with time until the maximum effect for the given concentration is achieved. For any fixed ethylene concentration in the air, plant response as a function of exposure duration will thus show a sigmoidal curve with a finite time lag. If we assume that a minimum concentration at the point of action is required in order to induce an effect on the plant, the lag period can be expected to be inversely related to the concentration of ethylene in the air. Thus, for experiments performed using a series of ambient concentrations, the observed plant response would consist of a series of parallel sigmoid curves with the lag period decreasing as ethylene concentration in the air increases (Woltering and Harkema 1987; Woltering et al. 1993).

For observations made at fixed exposure time under variable ambient ethylene concentrations, similar sigmoidal curves may also be expected. If the concentration were too low, there would not be a sufficient concentration of ethylene in the plant for the given time period and there would be no observable effect. At higher concentrations, the effect increases as the concentration increases. Again a sigmoidal response is expected. As exposure time increases, the sigmoidal response curve is expected to shift towards the lower concentrations, producing a series of parallel sigmoidal response curves as reported by Goeschl and Kays (1975), Woltering and Harkema (1987) and Woltering et al. (1993).

### Defining dose for ethylene exposure

As we have discussed above, at a given concentration, the effect is expected to increase as the duration of exposure increases. For each concentration, one may thus find a relationship between impact and duration of exposure. Different concentrations will result in a family of effect-duration curves. At the same time, for a fixed duration, the effect is expected to increase as the concentration increases. For a fixed duration, we may expect a relation between effect and concentration. For different durations, a family of effect-concentration curves, each corresponding to a fixed exposure time, is expected. The question that then needs to be answered is: can we find a general relationship between effect (response) as the dependent variable, the duration and concentration as independent variables?

For this, we look for a dose-response relationship, where "dose" must be defined to combine the contributions of both duration ( $t$ ) and concentration ( $C$ ) aspect of the exposure of plants to atmospheric ethylene.

$$[A-1] \quad \text{Dose} = f(C,t)$$

The general requirement is that the "response" is a non-decreasing function of the "dose", however the dose is defined. Thus, if yield loss is defined as the "response", yield loss is a non-decreasing function of the dose.

At any concentration, exposure increases with duration. Thus, dose at any concentration should increase as duration increases. On the other hand, for any fixed duration, dose should increase as concentration increases. Thus, we may require that the "dose", however it is defined, be a non-decreasing function of both the exposure duration and concentration.

There are many possible ways of defining "dose" as a function of concentration and duration that will satisfy these requirements. One of the simple definitions is:

$$[A-2] \quad \text{Dose} = f(t) g(C)$$

where  $f(t)$  and  $g(C)$  are both non-decreasing functions. The simplest definition is  $f(t) = t$  and  $g(c) = c$  so that the equation [A-2] becomes:

$$[A-3] \quad \text{Dose} = C t$$

Two problems arise from this definition: first, it suggests that if the duration of exposure were sufficiently long, any concentration above zero would produce a measurable effect. This is clearly not true based on experimental observations. A minimum concentration is thus introduced. It is assumed that at concentrations below  $C_0$ , no effect would be observed regardless of the length of exposure. Secondly, the definition suggests that for an arbitrarily short duration, if the concentration is sufficiently high, an effect would be observed. This may be true if the concentration is defined as the concentration inside the plant tissue at the point of action. However, if the concentration is taken as the atmospheric concentration, allowance must be made for the time lag described above.

Thus, dose is defined as a function of ethylene concentration and exposure time so that:

$$[A-4] \quad D = (C_{air} - C_0) f(t)$$

where  $C_{air}$  is concentration in the air,  $C_0$  is the minimum concentration required to induce an effect and  $f(t)$  is a function of exposure time.

Assuming that the lag takes a finite amount of time, the effect on the plant will require a minimum exposure length. Afterwards the effect will increase with increasing exposure time.

Assuming that dose is a function of gas concentration at the point of action in the plant tissue,  $C$ , if this concentration varies with time, the dose is defined by integration as:

$$[A-5] \quad D = \int_0^t (C - C_0) dt .$$

Assuming ethylene enters plant tissue by diffusion, the concentration at the point of action is given by (Crank 1992):

$$[A-6] \quad C - C_0 = (C_{air} - C_0) \left( 1 - erf\left(\sqrt{\frac{a}{t}}\right) \right)$$

where  $erf$  is the error function, defined as:

$$[A-7] \quad erf(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^x e^{-\frac{1}{2}z^2} dz$$

Equation [A-7] results from solution of the one dimensional diffusion equation from the surface to the point of action in the plant tissue. The parameter,  $a$ , can be viewed as the time lag that we have discussed above. Combining equations [A-5] and [A-6], the dose is thus defined as:

$$[A-8] \quad D = (C_{air} - C_0) \int_0^t \left( 1 - \operatorname{erf} \left( \sqrt{\frac{a}{t}} \right) \right) dt = (C_{air} - C_0) f(t)$$

where

$$[A-9] \quad f(t) = \int_0^t \left( 1 - \operatorname{erf} \left( \sqrt{\frac{a}{t}} \right) \right) dt$$

is the time function.

#### Selecting the parameters $a$ and $C_0$ in dose equation

One of the basic assumptions made here is that the response is a continuous function of dose. The best value for the time lag parameter,  $a$ , was computed using an iterative procedure. An arbitrary value, e.g.  $a = 1$  day, is first chosen. Doses for all treatment conditions were then calculated using equation [A-8] and the yield responses were plotted against calculated dose. The value of this parameter is adjusted repeatedly until the scatter of the data, calculated as the total sum of squared difference between data points and a multinomial regression equation, is minimized. A single lag time,  $a = 0.5$  day, is appropriate for all three crops. The minimum concentration  $C_0$ , is determined in a similar fashion. The minimum concentration that fits all three crops is 10 ppb. This is in agreement with data from the literature (Reid and Watson 1981, Abeles et al. 1992).

The sensitivities of the dose to the lag time,  $a$ , can be illustrated through the following figures. When  $a = 0.5$  day, the response of the barley yield to the dose is shown in Figure III-1.

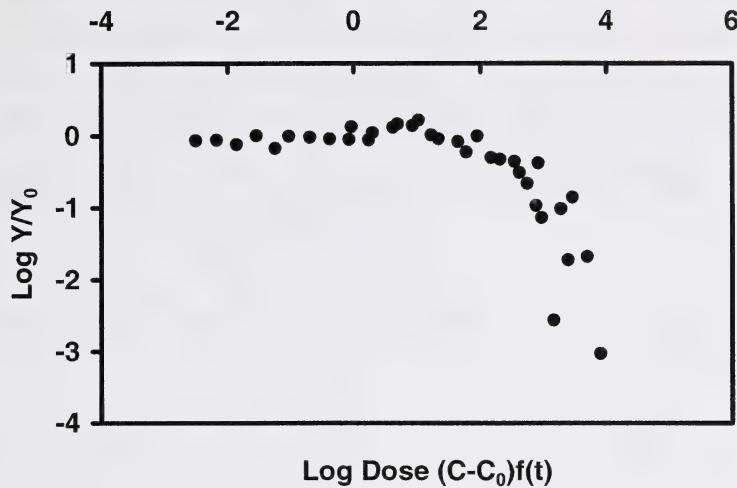


Figure III-1. The response of relative yield of barley to the dose function, with  $\alpha = 0.5$ .

When  $\alpha = 0$ , the lag time is zero and the dose function becomes: Dose =  $(C-C_0)t$ , the response of the barley yield to the dose is shown in Figure III-2. All the data concentrated in a small range of dose. It has little effect on the long-term exposure data but the short-term data is compressed. However, when  $\alpha = 2$ , the data scattered into groups (Figure III-3).

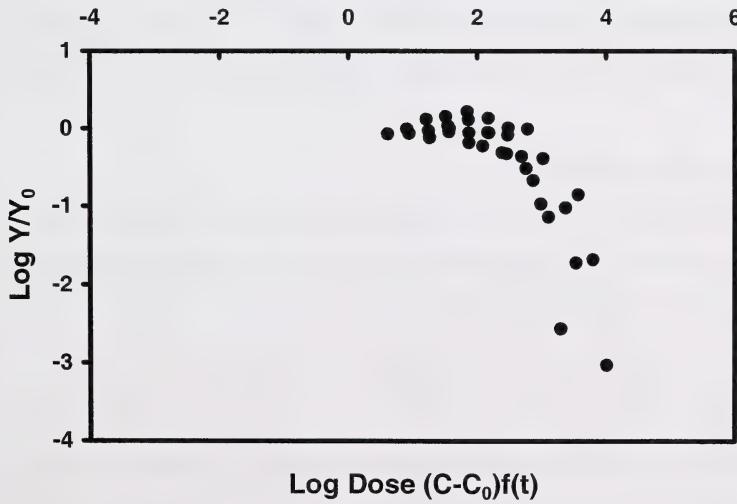


Figure III-2. The response of relative yield of barley to the dose with  $\alpha = 0$ .

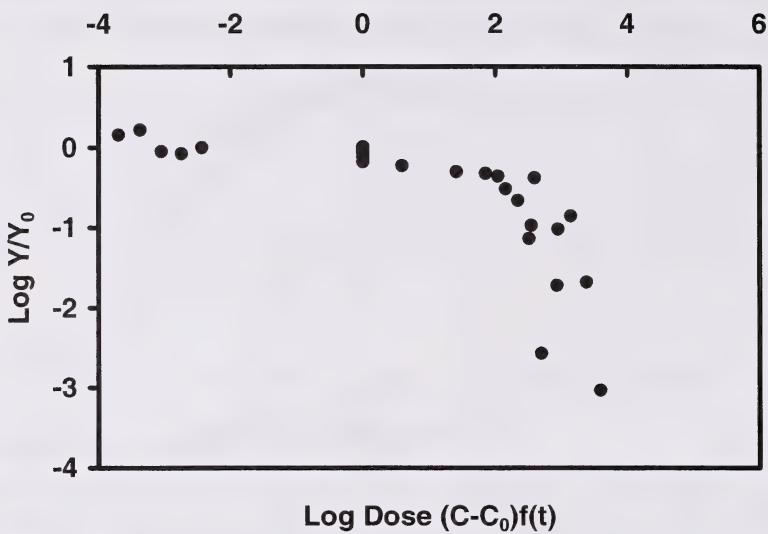


Figure III-3. The response of relative yield of barley to the dose function, with  $a = 2$ .

Ten ppb is the minimum background concentration in our experiments. Inclusion of the value does not affect the results extensively. When  $C_0 = 0$ , a nearly identical pattern is observed as the case of  $C_0 = 10$ . However, inclusion of  $C_0$  is going to affect the prediction of long-term responses. It predicts zero response when  $C < C_0$  when  $C_0 = 10$  is included however long the exposure. Without this, response is predicted for any  $C$ , if exposure is sufficiently long.

#### Developing a dose-response function

Using yield expressed as a percent of control (relative yield) as a measure of plant response and dose for ethylene exposure as defined in equation [A-8]. The experimental data was fitted into the following empirical dose-response function:

$$[A-10] \quad \text{Log} \frac{Y}{Y_0} = -\left(\text{Log} \frac{D_k}{D}\right)^{-n}$$

where  $Y/Y_0$  is the relative yield,  $D$  is the dose,  $D_k$  is the dose at which  $Y = 0$  and  $n$  is a dimensionless shape factor.

Equation [A-10] describes response,  $Y/Y_0$ , as a continuous function of dose. Under any practical situation, there are always uncontrollable factors that contribute to random variation of crop yield. One may argue that if the yield loss due to ethylene exposure is less than the random variation in crop growth, it will be undetectable and thus considered tolerable. Thus we define a maximum tolerable relative yield loss, or threshold response,  $\tau$ :

$$[A-11] \quad \frac{Y_\tau}{Y_0} = \frac{Y_0 - (Y_0 - Y_\tau)}{Y_0} = 1 - \tau$$

where  $Y_\tau$  is the yield when the threshold loss has occurred. Using this definition in equation [A-10], we have:

$$[A-12] \quad \log(1 - \tau) = -\left(\log \frac{D_k}{D_\tau}\right)^{-n}$$

where  $\tau$  is threshold relative yield loss,  $D_\tau$  is threshold dose. Assuming  $\tau \ll 1$ , we have:

$$[A-13] \quad \tau = 2.303 \left(\log \frac{D_k}{D_\tau}\right)^{-n}$$

or,

$$[A-14] \quad \frac{D_\tau}{D_k} = 10^{-(\tau/2.303)^{-1/n}}$$

Equation [A-14] shows that as  $n$  increases, the ratio  $D_\tau/D_k$  increases. For any threshold relative yield loss  $\tau$ , the corresponding threshold dose becomes smaller as  $n$  decreases. This means that the response is more sensitive to low doses with smaller  $n$ . The relationship between  $D_\tau/D_k$ ,  $\tau$ , and  $n$  is shown in Figure III-4.

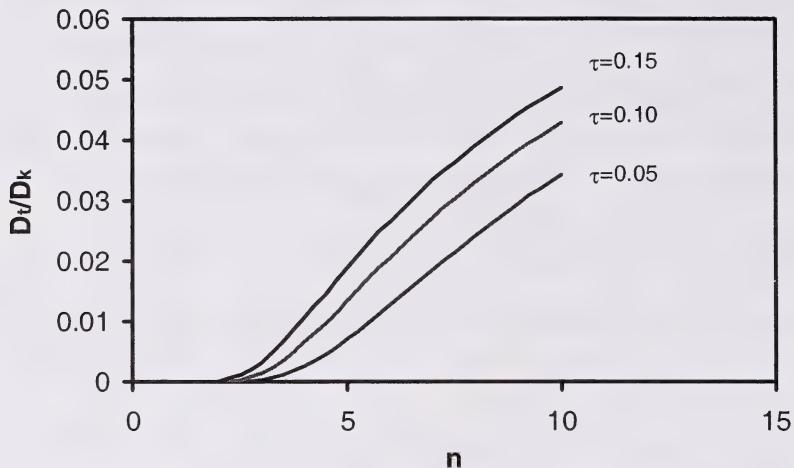


Figure III-4. Relationship between threshold yield loss,  $\tau$ , the dimensionless shape factor,  $n$ , and the threshold dose  $D_\tau$ .

The data for barley can be used as an example. Experimentally measured relative yields ( $Y/Y_0$ ) as a function of dose calculated using equation [A8] with the parameters  $a = 0.5$  day and  $C_0 = 10$  ppb are plotted in Figure III-5. These data include the results of both short- and long-term experiments.

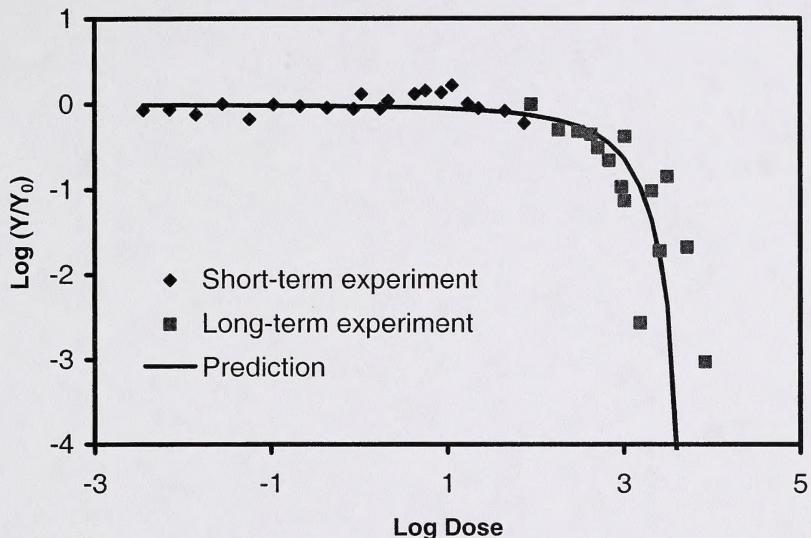
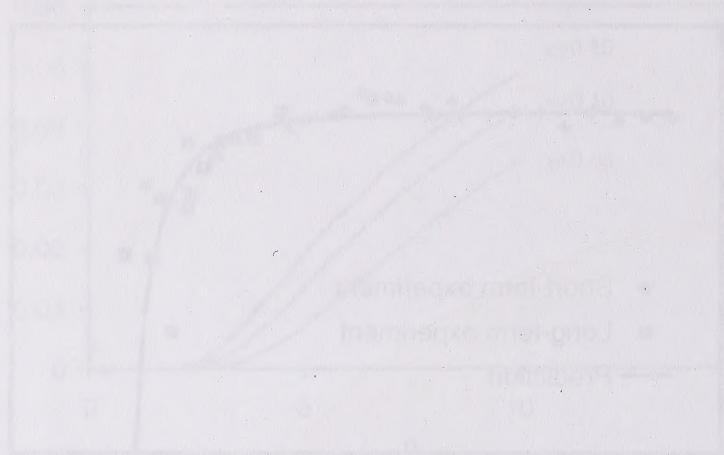


Figure III-5. Yield response of barley to exposure to ethylene.

The parameter values that best describe the data are  $n = 2.5$  and  $\text{Log}D_k = 4.2$ . At  $\text{Log}(\text{Dose}) < 1$ , there is no apparent decrease in yield. The coefficient of variance for these data is 0.25. If one assumes that the average yield loss threshold equals to 0.25, the threshold dose is, according to equation [A-14],  $\text{Log}(D_\tau) = 1.79$ . If, however, one requires the expected average yield loss to be less than 10%, the threshold dose becomes  $\text{Log}(D_\tau) = 0.69$ .



the field, and the other 20% of the area was covered with grassy soil.

The results of analysis of sand samples from the different areas will be

summarized below. Experiments conducted on the sand samples from the different areas will be described later.

Analyses of sand samples from the different areas were made by the Bureau of Soils, and the results are summarized below.

The analyses of sand samples from the different areas are summarized below. The results are summarized below.

Analyses of sand samples from the different areas are summarized below.



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